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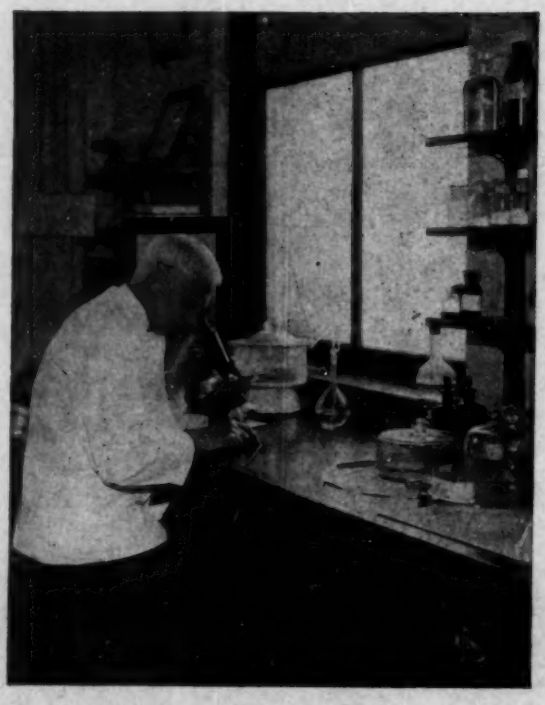

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The Journal of Laboratory and Clinical Medicine

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CLINICAL AND EXPERIMENTAL

ROUTINE SKIN TESTS IN CHRONIC NEPHRITIS*

BY LOUIS C. B. BALDWIN, M.D., BOSTON, MASS.

IN VIEW of the lack of fundamental knowledge concerning the etiology of chronic nephritis, it seemed desirable to do skin tests on a series of patients with this condition in order to determine whether or not a certain proportion of them were hypersensitive to some particular food protein. The possibility that protein hypersensitiveness might be the basis of renal injury is suggested by certain experimental evidence. It has been shown for instance, by Longcope,[1] that repeated subcutaneous shocking doses of horse serum or egg white in sensitized rabbits, so spaced and of such an amount that they only caused mild symptoms, were capable of producing definite kidney lesions in a large proportion of the animals. His conclusions were later confirmed by Boughton.[2]

Martin and Pettit,[3] whose work has been corroborated by Valerie-Radot,[4] obtained practically the same results, by feeding their rabbits exclusively on powdered milk and meat protein. On this diet the animals lived from two weeks to five months, and in some cases developed a rapidly fatal acute nephritis, and in others a more chronic form. These investigators made no attempt, however, to determine why a high protein diet causes kidney injury; they neither described the symptoms in the course of their experiments, nor apparently subjected their animals to subcutaneous doses of the offending protein, or in other manner attempted to determine the existence or non-existence of anaphylaxis.

Newburgh[5] was also able to produce nephritis in rabbits by feeding them egg albumen, casein, and soy bean. Unquestionably, therefore, a high protein diet causes kidney damage in rabbits, but the mechanism by which this is

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produced is still obscure. In animals fed on large amounts of egg albumen, Newburgh was unable to demonstrate precipitins in the blood for the ingested protein; consequently it would not appear that anaphylaxis was responsible for the nephritis in his animals.

Some more recent work of Bell and Hartzell[6] points out certain pitfalls one is in danger of when too close analogies are drawn between what occurs in laboratory animals under experimental conditions and what is found in man. They maintain in the first place that it is very difficult to be certain that experimental procedures are alone responsible for kidney lesions, and in the second place that a comparison is still further subject to question in view of the fact that there are marked differences between the lesions found in rabbits which have been subjected to repeated nonfatal poisoning with foreign protein and those found in man. In these animals, moreover, disease of the kidney is quite common and often of spontaneous occurrence, that is to say, independent of any intentional procedure carried out by the investigator. They conclude that there is no experimental evidence that foreign protein is in any way responsible for chronic nephritis in man.

In spite of their evidence to the contrary, it does not seem unlikely that hypersensitiveness may be at the bottom of certain cases of chronic nephritis in which no other ascertainable cause can be found. This applies particularly to those patients who present no antecedent acute infections and no histories of repeated acute exacerbations of their nephritis. Presumably in this type of case, sensitization resulting in renal injury might occur through the gastrointestinal tract. That proteins may, under certain conditions, be absorbed unchanged after ingestion, has been pretty definitely established by Schloss and Worthen,[7] Lust,[8] Moro,[9] and others, who have shown the presence of unaltered food proteins in the blood of some infants. It is not inconceivable that absorption of this kind might account for the manner in which some children become hypersensitive to proteins in their diet, though possibly sensitization through the gastrointestinal tract does not always account for hypersensitiveness, since there are exclusively breast-fed infants with eczema who give positive skin reactions with egg albumen, cow's milk or other proteins which they have never eaten. In order to explain sensitization on similar grounds in these cases one would have to assume that unaltered proteins were absorbed by the mother and excreted unchanged in her milk, so that the nursing infant could obtain the offending protein from that source. This point has been investigated by Shannon,[10] who claims to have shown the presence of food proteins in mother's milk by anaphylactic experiments on guinea pigs. Stuart,[11] however, was unable to verify his experiments in spite of the fact that he used more delicate methods for identifying the presence of food protein.

Granting that an individual is hypersensitive we are not vitally concerned in this investigation with how that condition was brought about. We know that foods are capable of causing symptoms of intoxication upon ingestion in hypersensitive individuals. An extreme case recently cited by Coca[12] is illustrative of this fact. In this individual swelling of the lips and tongue followed by a generalized pruritus would occur when cooked pea was taken into the mouth without being swallowed.

SELECTION OF CASES

The present series consists of 23 unselected cases of chronic nephritis. At first it was thought advisable to exclude the patients who gave a definite history of an acute infection immediately anteceding their nephritis because of the fact that in this type of case the renal injury seemed to be bacterial in origin. More careful analysis of their histories, however, made it apparent that one could never be sure in an individual instance that the infection was directly responsible for the nephritis. On the contrary, might one not be dealing with an acute exacerbation of a chronic process? In other words, was it not reasonable to suppose, that a preexisting condition was only being stirred up by the bacterial toxemia, and not initiated by it? In favor of this view is the fact that several of the cases gave no histories of an antecedent infection, and yet the course of their disease was interrupted in its progress by acute exacerbations, sometimes directly following an infection, at others apparently spontaneous in origin.

In addition to their renal history, the patients were carefully questioned as to whether they, or any members of their family, had had asthma, hay fever, urticaria, or chronic bronchitis. This was done for two purposes, to exclude the possible independent existence of two diseases in the same person, and to determine if protein idiosynerasies are more common in chronic nephritics than in normal individuals. One would expect that if nephritis were often caused by sensitization to protein, that some of the more generally recognized symptoms of protein sensitization would manifest themselves at some time in a certain proportion of the patients.

Each patient was further questioned as to whether there was anything of a dietary nature which he avoided, either because of an actual distaste or because it disagreed with him. This is an important symptom of intolerance to protein, particularly in children. One commonly finds that a mother has been unable to make her child eat eggs or drink milk, and subsequent investigation shows that the child is hypersensitive to the protein for which he has an active dislike and consequently refuses to eat.

SUMMARY OF CASES

CASE 1.—N. C., aged thirty-eight years. In October, 1918, had influenza, followed shortly by swelling of the extremities, headaches, dyspnea, weakness, and edema of the eyelids. Course very gradually progressive. Since 1919 urine showed a fixed low specific gravity, a heavy trace of albumin, moderate number of hyaline and granular casts. Blood pressure and B.U.N. gradually rose, pthalein dropped. Developed a terminal pericarditis. No history of protein sensitization.

CASE 2.—P. M., aged fifty-two years. Repeated attacks of acute articular rheumatism and erysipelas of face on several occasions, in 1917, acute nephritis immediately followed erysipelas. Since 1915 had had nocturia once. The functional tests showed a persistent nephritis with latterly a blood pressure between 140/84-156/98, phthalein between 34 and 48. The urine showed numerous casts and a fixed low specific gravity. No history of protein sensitization.

CASE 3.—H. C. B., aged thirty-four years, had scarlet fever in infancy. In 1916 had acute tonsillitis followed by nephritis. Suffered from severe headaches and weakness since 1917 when urine examination showed constant albumin and casts. Course gradually progressive. Latterly blood pressure over 200 systolic, phthalein below 20 per cent; B.U.N.

slightly elevated. Noteworthy persistence of red blood cells in the urine. No history of protein sensitization.

CASE 4.—H. G. B., aged twenty-three years. Following influenza in 1919 developed symptoms of acute nephritis. In 1921 because of persistent haematuria a double renal decapsulation performed without benefit. Renal functional tests persistently very slightly impaired. Haematuria persists with occasional periods of acute exacerbation. Has always disliked eggs. Eight months previous to having skin tests, had hives of two days' duration. No other history of protein sensitization.

CASE 5.—E. W., aged twenty-six years, has had numerous head colds for years. Much infection around the teeth since childhood. In 1917 complained of a gradual onset extending over a period of months, of failing vision, headache, and edema. At this time there was a well marked albuminuric retinitis. The blood pressure was 240/140 and the urine showed a trace of albumin and an occasional hyaline cast. Since then the course has been steadily, but very slowly progressive, lately complicated by an acute pericarditis. No history of protein sensitization.

CASE 6.—A. R. H., aged nineteen years, had several attacks of tonsillitis in childhood. In 1919, without known cause, puffiness of face and edema of lower extremities developed. Six months later had a mild attack of influenza followed by an acute nephritis. In 1921 another acute attack with general anasarca, edema of retina and discs, urinary retention, moderately elevated blood pressure, but good renal function. One month later had a similar but more severe attack with good renal function but with convulsions, Cheyne-Stokes respiration, and coma. Blood pressure at times 220/154. Numerous hemorrhages occurred in both eyes. Improvement set in gradually. Since then the progress has been steadily down hill, with a slow drop in renal function. No history of protein sensitization.

CASE 7.—W. J. D., aged sixty years, has had dyspnea, palpitations of the heart, and precordial pain on exertion for a number of years. Physical examination revealed an enlarged heart, accentuated A_2, and a blood pressure of 162/105. Phthalein elimination slightly impaired. Urine showed a very slight trace of albumin and rare graular casts. No history of protein sensitization.

CASE 8.—R. A., aged twenty-seven years. History of nephritis for ten months. Acute symptoms developed immediately following an extensive, reddened, scaly eruption of the face. Course rapidly down hill with a progressive impairment in renal function. No history of protein sensitization.

CASE 9.—B. M., had a chronic cough for years. Tonsillitis and scarlet fever in childhood. Following the scarlet fever had dizziness, blurred vision and nausea, also edema of the lower extremities. Since primary attack these symptoms have recurred at intervals, and for several years the patient has had nocturia. Had convulsions during two pregnancies, which had to be terminated. Renal function not depressed. Urine showed moderate to numerous hyaline casts, some with fat, rare to moderate red blood cells. No history of protein sensit'. ation.

CASE 10.—H. B. D., aged twenty years, had diphtheria eight years ago. For seven years has had headaches and nose bleeds. Four years before noticed puffiness of the eyelids. Lately has felt tired and weak. Except for slight edema, physical examination negative. Renal functional tests normal. No elevation of blood pressure. Urine shows slight trace of albumin casts, and persistent red blood cells. No history of protein sensitization.

CASE 11.—H. B., aged nineteen years, has had no past illnesses. Six months before began to suffer from malaise and a general tired feeling. Five and one-half months before edema of eyelids appeared. Aside from large tonsils and edema of eyelids and lower extremities, physical examination essentially negative. Renal function very slightly depressed at times. Blood pressure occasionally a trifle elevated. The urine showed a trace to a large trace of albumin, numerous fatty and granular casts, rare to numerous red blood cells. No history of protein sensitization.

CASE 12.—A. L. H., aged thirty-eight years. Acute nephritis a year before following pneumonia. Since then the urine has persisted to show albumin, casts and red blood cells. Renal function is good, blood pressure slightly elevated at times. No history of protein sensitization.

CASE 13.—I. M., aged fourteen years. Pneumonia twice, once in infancy and once three years before. Tonsillitis several times, the last attack quite recently. Patient felt well until a year and a half before, when, without apparent cause, she noted general weakness and shortness of breath on exertion. In September of the same year, following a severe cold, her face became very puffy. There were no other symptoms of nephritis. Examination showed a general anasarca with secondary anemia. The urine contained albumin, casts of all descriptions, and moderate to numerous red blood corpuscles. The blood pressure was 140/100. There was no impairment in renal function. The course has remained stationary except for the fact that there have been periods in which the edema has entirely subsided. No history of protein sensitization.

CASE 14.—M. B., aged thirty-four years. Diphtheria at two years. A year before began having swelling of ankles which soon spread upward to the legs and thighs. Except for moderate shortness of breath on exertion felt quite well. The urine showed albumin and casts. Renal functional tests showed a slight impairment at first but none lately. There was no elevation in blood pressure. Upon an Epstein diet the edema almost entirely cleared up, and patient felt much better. No history of protein sensitization.

CASE 15.—S. S., aged forty-nine years. Slight palpitation of the heart and dyspnea on exertion, swelling of the legs, and nocturia for three years. Seven months before admission to hospital began to suffer from dizziness, blurred vision, nervousness and insomnia. This was at the time of her last menstrual period. Physical examination showed a moderately enlarged heart with a blowing apical systolic murmur, slight pitting edema of the shins, and sclerosed vessels. The blood pressure was 205/120. There was no impairment in renal function, and the urine was negative. This apparently is a case of vascular hypertension and not of nephritis. No history of protein sensitization.

CASE 16.—F. T., aged thirty years. Diphtheria at six years of age, mild influenza in 1918. In June, 1920, had feet strapped to support fallen arches. Noticed at this time swelling of feet and ankles. No other symptoms. The renal functional tests remained normal at all times. There was no elevation of blood pressure. The urine showed considerable albumin, hyaline and granular casts, some fatty casts, free fat and red blood cells. Course on the whole was stationary. Lately there has been a recurrence of edema. No history of protein sensitization.

CASE 17.—T. W. B., aged thirty-two years, has had asthma for fifteen years; also for several years hay fever coming on in September and remaining until the first frost. Gradual onset seven months before with general weakness, and mild edema of the ankles. No antecedent infections. Blood pressure and renal functional tests normal. The urine showed a persistent trace of albumin, numerous hyaline casts, rare to moderate granular and few fatty casts, also rare to numerous red blood cells and white blood cells.

CASE 18.—H. C., aged twelve years, repeated attacks of sore throat. One and one-half years ago suddenly developed edema of the face and lower extremities. Vomited for a week and had several convulsions. At this time the urine showed numerous granular and hyaline casts and was of low fixed specific gravity; the blood pressure was 200/130. A year later the child had an acute attack of tonsillitis accompanied by renewed signs of active destruction of the kidneys. The course has been very gradually progressive. No history of protein intolerance.

CASE 19.—S. C., aged forty-five years, always well up to fourteen months before, when two to three days following an attack of grip hematuria and swelling of the lower extremities were noticed. Upon rest in bed the swelling and hematuria gradually disappeared. She resumed her housework and felt quite well until three months before when

the swelling of her lower extremities returned, and her abdomen began to enlarge. The blood pressure was 162/100, the phthalein 10 per cent, the urine showed a trace of albumin and numerous hyaline and granular casts. No history of protein sensitization.

CASE 20.—J. P., aged forty years. Measles and smallpox early in life. Two years before, patient caught cold while bathing and developed a severe, productive cough. At the same time became short of breath on exertion, this persisted until six weeks before admission to the hospital when, in addition to the cough, she complained of a loss of appetite and swelling of the feet. For two years she had had nocturia. The urine persistently showed signs of active kidney destruction. Her condition grew rapidly and progressively worse. She died in uremic coma. No history of protein sensitization.

CASE 21.—C. H., aged twenty-five years. Diphtheria in childhood, and tonsillitis every winter until tonsillectomy three years before. Thirteen months before admission, during sixth pregnancy, began having failing vision and suboccipital headaches. Two months later convulsions set in, and patient had a premature labor. Was better for a time then gradually got worse. B.U.N. increased, phthalein depressed. Urine showed a large trace of albumin and numerous casts of all descriptions. No history of protein sensitization.

CASE 22.—M. C., aged forty-three years. Childhood diseases, including scarlet fever. During pregnancy fifteen years before had general anasarca, headache, marked albuminuria. Another pregnancy six years before resulted in a miscarriage. Has had nocturia 1 to 3 times for fifteen years. Also mild, occasional puffiness of the ankles and frequent headaches for fifteen years. For two years has been short of breath upon the slightest exertion, and for seven months her eyesight has been failing. Retinal examination revealed a marked albuminuric retinitis. The B.U.N. was normal, the phthalein slightly depressed. The urine showed a large trace of albumin and numerous hyaline and granular casts. No history of protein sensitization.

CASE 23.—J. P. M., aged forty-eight years. Occasional mild sore throat for years, and measles and typhoid in childhood. Was examined and passed for life insurance ten years before. One year before began having nocturia 2 to 4 times, otherwise felt perfectly well. Four months before, was refused an increase in his life insurance on account of albumin in the urine. Three weeks before began having trouble with his eyes, the sight failing rapidly since. Physical examination showed marked arteriosclerosis with hypertension, and a severe retinitis. Renal functional tests depressed. The urine showed considerable albumin, hyaline and granular casts, and red blood cells. No history of protein sensitization.

PROTEINS EMPLOYED FOR THE SKIN TESTS

Only the more common food proteins were employed for skin tests, since it seemed reasonable to assume that if chronic nephritis were the result of sensitization of dietary origin, substances which were frequently eaten could alone be held responsible for the renal injury; for it would be difficult to conceive how a condition, presumably dependent on repeated protein reactions could be caused by a protein only rarely eaten.

The same proteins were employed for the skin tests in every case. They were applied cutaneously by the Walker technic. That is to say, small cuts were made in the skin of the forearm, just deep enough to penetrate the superficial layers but not to draw blood. A protein in powdered form was placed on each of these cuts except one, and a drop of decinormal sodium hydroxide added to act as solvent. From time to time the sites were moistened with the sodium hydroxide, and at the end of half an hour the proteins were washed off and readings made. For control a cut was made, upon which decinormal sodium hydroxide alone was placed. In interpreting re-

sults, a positive reaction was considered as one consisting of a wheal, at least 0.5 cm. in diameter, surrounded by a variable zone of erythema, while if there were only a zone of erythema of from 15 to 20 mm. in diameter, but no wheal, the reaction was termed doubtful. Of course, a comparison with the control site is important in every case, but particularly in cases of doubtful reactions, since the latter resemble closely nonspecific irritative phenomena. Consequently, whenever a doubtful reaction was obtained, it was repeated on a fresh site that same day and on a subsequent day. Only if the reaction was repeatedly obtained, was it recorded as of possible significance. The tested sites were again inspected at the end of twenty-four hours for the possible development of delayed reactions.

FOOD PROTEINS EMPLOYED CUTANEOUSLY

1. Wheat globulin	7. Rice	13. Lact Albumen	19. Chicken
2. Wheat glutenin	8. Potato	14. Casein	20. Halibut
3. Wheat gliadin	9. Pea	15. Cocoa	21. Codfish
4. Oat	10. Bean	16. Beef	22. Salmon
5. Barley	11. Tomato	17. Lamb	
6. Rye	12. Egg white	18. Pork	

Since a number of the patients had suffered from repeated attacks of tonsillitis, or had infected teeth, it was thought advisable to test them also with bacterial vaccines of the more common mouth organisms with the view that possibly they might show a sensitization to some bacterial protein. For this purpose the more delicate intracutaneous method was employed because the vaccines which we used for the purpose were fairly dilute when compared with the proteins applied in powdered form, and consequently it was thought that a mild positive reaction might appear by this method when the less delicate cutaneous application would fail to react. Briefly, the arm was cleansed with alcohol, and about 0.05 c.c. of the vaccine was injected intradermally with a very fine needle. The size of the initial wheal was noted, and a final reading was made at the end of half an hour and again in twenty-four hours. When an autogenous vaccine had been made from the patient's tonsils or infected tooth socket, a skin test was also performed with the latter.

VACCINES EMPLOYED FOR INTRADERMAL TESTS

Streptococcus pyogenes	Streptococcus mitis	Streptococcus fecalis
Streptococcus infrequens	Streptococcus salivarius	Staphylococcus aureus
Streptococcus anginosus		

RESULTS

The accompanying chart shows that only two cases gave definitely positive reactions to food proteins. Case 4 had been unable to eat eggs from childhood because he disliked them and because he had learned from experience that they disagreed with him, causing considerable epigastric distress. On one occasion, a year previous to doing the skin tests, he had had a mild attack of urticaria lasting two days, but otherwise his history was negative regarding protein hypersensitiveness. There was a mild reaction to egg white in this case. Upon reviewing the history of his nephritis, one sees that the onset was sudden, following an attack of influenza, and the subsequent course

CHART I

PATIENT	PREVIOUS ILLNESS	ANTECEDENT INFECTION	DURATION	HISTOLY OF PEOTEIN ALLERGY	FOOD PROTEINS		BACTERIAL VACCINES		AUTOGENOUS VACCINE
					POSITIVE	DOUBTFUL	POSITIVE	DOUBTFUL	
1	influenza	influenza	4 yr.	none	0	0	0	0	
2	rheumatic fever, erysipelas	erysipelas	5 yr.	none	0	0	not done	0	
3	scarlet fever, tonsillitis	tonsillitis	5 yr.	none	0	0	0	Staph. aureus	0
4	influenza	influenza	3 yr.	always disliked eggs. Hives on one occasion for two days	egg white +	0	0	Staph. aureus	
5	numerous head colds, much infection around teeth	none	5 yr.	none	0	0	0	Staph. aureus	
6	repeated tonsillitis in childhood	none	3 yr.	none	0	0	0	0	
7	negative	none	5-6 yr.	none	0	0	not done	0	
8	eczema of face		1 yr.	none	0	0	0	0	
9	chronic cough, tonsillitis, scarlet fever	scarlet fever	15 yr.	none	0	0	0	Staph. aureus	
10	diphtheria	possibly diphtheria	7 yr.	none	0	rice	0	0	0
11	none	none	8 mo.	none	0	0	0	Strep. infrequens	0
12	pneumonia	pneumonia	2 yr.	none	0	0	0	0	
13	pneumonia twice, tonsillitis several times	severe cold	2 yr.	none	0	rye	0	0	
14	diphtheria	none	1½ yr.	none	0	0	0	Staph. aureus	
15	none	none	3 yr.	none	0	0	not done	0	
16	diphtheria	none	2 yr.	none	0	pea	0	0	0
17	asthma	none	9 mo.	asthma 15 years hay fever for years	barley ++, oat +, rye +, ragweed +, wheat globulin +	wheat gliadin wheat glutenin 0	0	0	
18	repeated tonsillitis	none	1½ yr.	none	0	0	not done	0	
19	negative	influenza	14 mo.	none	0	0	not done	0	
20	measles, small pox	cold	2 yr.	none	0	0	0	0	
21	diphtheria, frequent tonsillitis	none	10 mo.	none	0	0	0	0	
22	scarlet fever	none	15 yr.	none	0	barley	0	0	
23	typhoid, occasional sore throat	none	1 yr.	none	0	0	0	0	

is rather remarkable in showing a persistent fairly severe hematuria. An intracutaneous test with Staphylococcus aureus gave a doubtful reaction. In Case 7, a man of thirty-two years of age, there was a history of asthma of fifteen years standing. This patient claimed that his first attacks occurred while working in a stable, and were accompanied by sneezing, running of the eyes and nose. At first his attacks were closely associated with stable work, though the patient was inclined to blame oats rather than horses for his condition, since the handling of oats always brought on an attack. Two years after his onset his asthma occurred at any time, but particularly at night and during damp weather. For several years he has also had hay fever, coming on regularly in September and lasting until cold weather. Cutaneous tests in this case were strongly positive with barley, oat, rye, and somewhat less strongly with wheat and ragweed. The nephritis was of gradual onset and of only seven months duration. These two cases were the only ones that gave histories suggesting hypersensitiveness to food proteins and they were the only ones that gave positive reactions with food proteins. Four patients gave doubtful reactions, Case 10 to rice, Case 13 to rye, Case 16 to pea, and Case 22 to barley.

Intracutaneous tests with the bacterial proteins were entirely unsatisfactory. Of the 18 cases tested, none gave positive results. There were 6 cases in which the reactions were doubtful, 5 of these with Staphylococcus aureus, and 1 with Streptococcus infrequens. Four cases were tested with their autogenous vaccines, but even with the bacterial vaccines obtained from their teeth or tonsils negative results were obtained. Case 3 gave a doubtful reaction with Staphylococcus aureus but negative with his autogenous vaccines which were respectively, Streptococcus mitis from an infected tooth and Streptococcus pyogenes from the tonsils. Case 11 reacted doubtfully with Streptococcus infrequens, but gave no reaction with his autogenous vaccine, which was a Streptococcus fecalis recovered from the tonsils.

CONCLUSIONS

From this series one can draw no conclusions. If the skin test is an accurate index of protein sensitization, it would seem that chronic nephritis is not commonly caused by proteins derived from the food. But on the other hand, there is abundant evidence elsewhere in the literature to show that protein sensitization may exist in spite of repeated negative skin tests, and therefore this problem will have to be studied further before any decision can be made.

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BLOOD URIC ACID IN NEPHRITIS*

By William Paul Holbrook, M.D., and Howard Davis Haskins, M.D.,
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THE aim of the work reported in this paper was to determine the clinical value of estimations of the uric acid of the blood as an indication of kidney impairment. For this purpose a study was made of 138 hospital cases, including estimations of urea, creatinin, uric acid and hemoglobin of blood, urine examination, functional tests and blood pressure, in addition to noting the age, history and clinical observations. Fifty-one of these cases were not used in preparing this paper either because they proved to be nonnephritic or because the nephritis was complicated by other conditions.

We have been interested in uric acid determinations for several years, chiefly because of the numerous statements in the literature that uric acid is excreted by the kidney with greater difficulty than urea or creatinin, and that, therefore, its retention gives the earliest indication of renal impairment. Myers, Fine and Lough[1] state that uric acid retention occurs before urea or creatinin are increased. Baumann, Hansman, Davis and Stevens[2] believe that uric acid determination is probably the most delicate index of renal function. Upham and Higley[3] claim that uric acid is the most difficult of all the waste products to excrete. Myers and Killian[4] also confirm that opinion.

We suspected that the current methods of estimating uric acid might be unsatisfactory; so we made a careful study of this question first. This work is reported in a recent paper.[5] We compared the results on 55 normal blood specimens by three methods, namely, the silver lactate and zinc chloride precipitation methods and the direct method. None of the methods were entirely satisfactory for a uric acid content of 1.5 to 3.7 mg. per 100 c.c. of blood, the direct method being the worst of the three. We were able, however, to introduce a modification into each of the methods so that they yielded good results. On trying these three modified methods with 25 hospital bloods, as well as with normal bloods, we secured very good results that show little variation by the different methods. Having determined how to secure accurate uric acid estimations, we were in a better position to make an intelligent study of the clinical value of such estimations.

After eliminating all cases in which the blood chemistry findings might be due in part to some condition besides a definite kidney lesion, there remain 87 cases of nephritis of various types. No cases are included in which metabolic disorders play any part. A summary of the results arranged in the order of the urea nitrogen content of the blood is given in Table I. Most of the results (i. e., up to 96 mg. urea N) are shown graphically in Chart 1, enabling one to

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see at a glance the comparative level of the uric acid, creatinin and urea nitrogen content of the blood in these cases. The line drawn at 16 mg. urea N, is the dividing line between normal figures and figures indicating retention. This line represents 4 mg. of uric acid and 1.6 mg. of creatinin.

DISCUSSION

The 12 cases that show no retention consisted of (1) cases of focal embolic nephritis (blood and casts in the urine, and a definite focus of infection), (2) several cases of early acute nephritis, and (3) cases of hypertension (trace of albumin and occasional casts in the urine).

TABLE I

UREA NITROGEN, URIC ACID AND CREATININ CONTENT OF NEPHRITIC BLOODS

NUMBER OF CASES	RANGE OF UREA N AS MG.	RANGE OF URIC ACID MG.	RANGE OF CREATININ MG.	NO. CASES HAVING PATHOL. UREA N	NO. CASES HAVING PATHOL. URIC ACID	NO. CASES HAVING NORMAL URIC ACID	NO. CASES HAVING PATHOL. CREATININ	NO. CASES HAVING NORMAL CREATININ
12	8– 15	1.2– 3.3	1.2–1.4	none	none	12	none	12
26	16– 24	1.7– 4.0	1.2–2.3	26	none	26	6	20
29	25– 50	1.5– 5.4	1.3–3.1	29	9	20	26	3
20	51–367	2.2–13.3	2.3–9.9	20	17	3	20	none
87				75	26		52	

Urea retention in 86 per cent of the cases. Creatinin retention in 60 per cent of the cases. Uric acid retention in 30 per cent of the cases.

In the first group of cases that show pathologic findings the urea N content is from 16 to 24 mg., which is the range of early retention. Not a single one of the 26 cases shows uric acid retention. In the next group which comprises the cases showing moderate retention, only 9 out of the 29 had uric acid retention in addition to the urea retention. In the group of marked retention (51 to 367 mg. urea N) 3 of the uric acid findings were normal. In these 3 cases with normal uric acid content the lowest creatinin estimation was 2.7 mg. and the lowest urea N was 62 mg. This failure of uric acid retention, when marked retention was indicated by the content of other substances, is difficult to understand, except possibly on the supposition of diminished production of uric acid.

On the other hand, there was retention of creatinin in 46 of the 49 cases in the last two groups (25 mg. or more of urea N). The 3 cases having normal creatinin gave urea N estimations below 30 mg. The general tendency toward increase of creatinin, paralleling distinct increase of urea N, is well shown in Chart 1.

Summarizing the 87 cases of nephritis, 86 per cent showed urea retention, 60 per cent showed creatinin retention, and only 30 per cent showed uric acid retention. The latter results are inconsistent with the idea that uric acid is the first substance to be retained in consequence of damage to the kidneys. In our series of cases uric acid estimations have given no information of diagnostic or prognostic value that was not given in a more reliable way by the urea and creatinin estimations.

As has been pointed out by others, creatinin estimations are valuable in certain cases as an aid to prognosis. Thirteen of our cases gave creatinin estimations of 4.9 mg. or more. Twelve of these have died. The remaining one has

recovered and shows no retention. It was in all probability a case of sympathetic anuria following nephrectomy. The retention disappeared in a few days.

We consider creatinin estimation valuable also as a check on urea estimation, particularly in cases of marked retention. The curve for creatinin content runs roughly parallel to the urea curve (above 25 mg. urea N), as shown by Chart 1. In contrast with this point it will be noticed that the curve for uric acid shows no relation whatever to the other curves.

Estimations of sodium chloride were made in all the cases, but we do not report them because we have been unable to attach any significance to them. The

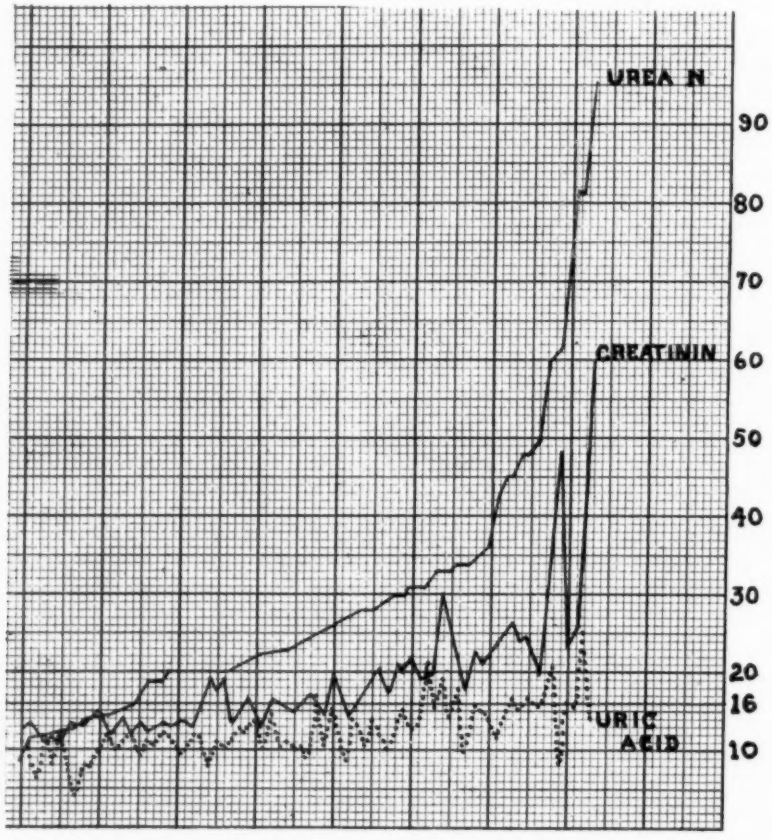

Chart 1.—Curves for the Creatinin, Uric Acid and Urea N Content of Nephritic Bloods. The line dividing normal and pathologic findings represents 16 mg. urea N, 4 mg. uric acid, and 1.6 mg. creatinin. The urea content at any particular point on the uppermost curve can be read by referring to the figures in the margin. To read the creatinin content from the middle curve the marginal figure must be divided by 10. The uric acid content is found by dividing the marginal figure by 4.

estimations seemed to be of no clinical value until they rose to nearly 600 mg. per 100 c.c. of blood. Very few of the cases gave an estimation as high as that even with marked retention of urea and creatinin. No difference in chloride content was observed in cases with edema and without edema.

The question of the range of normal findings for a particular constituent of blood is quite vital in blood chemistry work. From a large series of estima-

tions we conclude that urea N normals run from 7 to 15 mg. per 100 c.c. of blood, and that more estimations are near 8 mg. than are near 14 mg. All results above 15 mg. seem to us to call for attention clinically. In a previous paper[5] we reported the range of uric acid in 55 normal persons as 1.5 to 3.7 mg., but it is probable that the results are not clinically significant until 4 mg. is reached. The range of creatinin normals is in our opinion much lower than is ordinarily stated, probably 1 to 1.6 mg. We have not secured an estimation above 1.6 mg. in normal or in nonnephritic individuals. We found a wide variation of sodium chloride content in normal bloods, running from 316 to 550 mg. per 100 c.c.

Attention has been called to the possible effect of diet (exogenous purins) on the uric acid content of the blood. This was not a factor in the cases reported in this paper, since all of the 87 nephritics were in the hospital on a low protein diet.

It may be asked, how was it that others secured pathologically significant uric acid estimations in the earliest stage of retention while we had only one such estimation in all the cases showing less than 30 mg. of urea N? The only suggestion that occurs to us is that all of that previous work was done before the observation was made that starvation[6] results in distinct increase of blood uric acid. It is apparent now that there is a relationship between ketosis and increased uric acid content of the blood. This has been emphasized in a recent paper[7] on the effect of high fat diets. We consider it possible, therefore, that the previous uric acid findings, which were interpreted as indicating retention, were really due to dietary factors that were not recognized at the time.

Methods.—It seemed desirable to make all estimations on the protein-free filtrate prepared by Folin's tungstic acid method. A smaller amount of blood suffices for carrying out the methods. It gives greater uniformity in handling bloods, also greater leeway in doing the laboratory work because the filtrate keeps satisfactorily when saturated with toluol.

Urea N estimation.—Our standard method consists in warming 4 c.c. of oxalated whole blood mixed with urease and buffer phosphate for thirty minutes, adding potassium carbonate reagent, and by aeration drawing the ammonia over into 25 c.c. of N/70 acid. After titration of the acid left unneutralized, the urea N is easily calculated. This method must be started while the blood is still fresh. It was difficult at times to find opportunity to run the estimation promptly, also in some cases 4 c.c. of blood could not be spared.

Folin[8] suggests a method using a small amount of blood filtrate in which the final step is nesslerization. Never having been convinced that nesslerization and colorimetric estimations of ammonia are desirable or necessary, we have changed Folin's method so as to permit of titration.

Technic.—Measure 10 c.c. of blood filtrate into a large pyrex test tube and add 1 c.c. each of urease solution and buffer solution. Warm in a water-bath at about 55° C. for fifteen minutes. Add 5 drops of caprylic alcohol, two glass beads, and 2 c.c. of saturated borax solution. Connect at once with the condenser (we use a Hopkins' bulb fitted to the pyrex tube with a rubber stopper and a 25 c.c. pipette as a delivery tube attached to the free end of the bulb).

Add 5 c.c. of N/70 acid, 5 c.c. of water and 1 c.c. of 0.05 per cent sodium alizarin sulphonate solution to a short test tube (1 inch diameter), and then adjust the tip of the apparatus so that it is in the liquid. Heat with a small flame from a microburner. About four minutes' boiling is generally required, but continue the distillation for a full minute after steam comes over. During the last half minute keep the tip of the delivery tube raised slightly above the acid solution (we lower the test tube by the use of blocks). Rinse off the tip with a little distilled water. Titrate the distillate mixture with N/350 sodium hydroxide.

Calculation.—Subtract the c.c. used for titration from the titration figure for the control (run this control once a day using urease and all reagents but no filtrate), then multiply by 4, the result is milligrams of urea N in 100 c.c. of blood.

Reagents.—(1) Urease solution: grind up a tablet of urease (0.1 gm.) in a mortar and mix thoroughly with 5 c.c. of water. (2) Buffer solution: dissolve 2.8 gm. of sodium pyrophosphate in 100 c.c. of N/10 phosphoric acid. (3) N/350 sodium hydroxide: prepare a stock solution of N/35 NaOH and keep it in a nonsol or pyrex flask, from this prepare *each week* a supply of N/350 solution by making a 1 in 10 dilution.

Note.—The method was checked against the standard whole blood method by running both methods on the first 34 cases (urea N varying from 8 to 200 mg.). The variation in the results by the two methods was small, and in many cases they agreed as closely as duplicates by one method. The filtrate method was, therefore, adopted for the rest of the cases, checked occasionally by estimation by the standard method.

Uric acid estimation.—The direct method (Folin-Benedict) as modified by us[5] was used. It is necessary to repeat the warning given in our previous paper[5] that the direct method as ordinarily carried out is very unsatisfactory when the concentration of uric acid is below 4 mg. With our modification, however, which consists in adding 0.5 c.c. of pure uric acid solution (equivalent to adding 2 mg. of uric acid to 100 c.c. of blood) to 5 c.c. of blood filtrate before adding Folin's sodium cyanide and phosphotungstic reagents, the color that is developed is satisfactory for estimation, and the results are quite accurate. When there is a variation from the check method (precipitation by silver or zinc) it is generally on the side of slight excess. For research accuracy a check method should be run, we believe, whenever the direct method gives results above the normal content of the blood.

Recently Benedict[9] and Hunter and Eagles[10] have reported the isolation of a new substance from blood which gives a blue color with the uric acid reagents. This compound is supposed to be responsible for the occasional excess estimation by the direct method as compared with that by the check method. Apparently the occurrence of this substance in hospital bloods is infrequent. It is possible that Folin's improved phosphotungstic reagent may not react to this substance, so that the new reagent should be used by preference. The color developed by the aid of this reagent is better for estimation than that produced by Folin's older reagent. The latter, however, is fairly satisfactory.

Creatinin estimation.—Folin's method[12] was used. Measured portions of blood filtrate and of standard creatinin solution are treated simultaneously with freshly prepared reagent made by mixing picric acid solution with sodium hydroxide and are then compared in the colorimeter.

Sodium chloride estimation.—Whitehorn's method[13] was used. The Cl of blood filtrate is precipitated with standard silver nitrate and the excess of silver remaining in solution is titrated with sulphocyanate using ferric alum as the indicator.

CONCLUSIONS

1. Uric acid determinations cannot be used clinically as a reliable indication of kidney impairment. Uric acid is not retained in the blood at an earlier stage of nephritis than is urea.

2. Creatinin estimation is much more valuable, agreeing approximately with the urea results and serving as a check on the latter. High estimations are very significant for prognosis.

3. Urea estimations are the most reliable and significant of the blood chemistry findings in nephritis. Estimation of urea in blood filtrate by Folin's method using titration instead of nesslerization is very advantageous.

4. Sodium chloride estimation yields such variable results that its value in nephritis is very doubtful.

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REDUCING SUBSTANCES IN THE URINE*

By R. D. Leas, M.D., Cleveland, Ohio

THE subject matter in this report is not new material but is presented with the idea of reviewing a few well-known but often forgotten facts.

It has many times been recorded that the administration of salicylates will give a reducing substance in the urine, which cannot be differentiated from sugar by the reduction test alone. Other substances giving a similar reaction are uric acid, creatinine, simple aldehydes, formalin and chloroform. Occa-

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TABLE I*

CASE NO.	DATE	DRUG	AMOUNT OF DRUG	REDUCING SUBSTANCE IN URINE
1	12/27/25	0	0	0
	12/28/25	Sod. Sal.		0
	12/29/25	" "	540 gr.	0.50%
	12/30/25	" "		0
2	12/23/25	Thymol	?	0.316%
	12/24/25	Cinchophen		0.150%
	12/25/25	"		0
	12/26/25	"	500 gr.	0.260%
	12/27/25	"		V. F. T.
	12/28/25	"		0.208%
	12/29/25	"		0.277%
	12/30/25	"	0	Less than 0.150%
3	12/17/25	0	0	0
	12/18/25	Cinchophen	210 gr.	V. F. T.
	12/19/25	"		F. T.
	12/20/25		0	0
	12/21/25		0	–
	12/22/25		0	Less than 0.15%
	12/23/25		0	V. F. T.
	12/24/25		0	0
	12/25/25		0	0
	12/26/25		0	0
	12/27/25		0	0
	12/28/25		0	0
	12/29/25		0	Less than 0.15%
	12/30/25		0	V. F. T.
	12/31/25		0	0
4	12/11/25	0	0	0
	12/11/25	Cinchophen	150 gr.	0
	12/12/25	"		0.227%
	12/13/25	Sod. Sal.	640 gr.	V. F. T.
	12/14/25	" "		0
5	12/13/25	0	0	0
	12/13/25	Cinchophen		0
	12/14/25	"		0
	12/15/25	"	240 gr.	0
	12/16/25	"		V. F. T.
	12/17/25	"		Less than 0.15%
6	12/14/25	0	0	0
	12/14/25	Cinchophen		0
	12/15/25	"		0
	12/16/25	"		0.208%
	12/17/25	"	240 gr.	Less than 0.15%
	12/18/25	"		" " 0.15%
	12/19/25	"		0
	12/20/25	"		0.250%
	12/21/25	0		0.316%
	12/22/25	Sod. Sal.		0.380%
	12/23/25	" "		0.430%
	12/24/25	" "		0.330%
	12/26/25	" "		0.250%
	12/27/25	" "	1760 gr.	0.227%
	12/28/25	" "		0.150%
	12/29/25	" "		Less than 0.150%
	12/30/25	" "		" " 0.150%
	12/31/25	" "		0
	1/ 1/26	Tolysin	Gr. XV q.2h.	0
	1/ 2/26			0
	1/ 3/26			Less than 0.150%

*I wish to thank George Thorngate, M.D., for his aid in securing the data in this table

TABLE I—CONT'D

CASE NO.	DATE	DRUG	AMOUNT OF DRUG	REDUCING SUBSTANCE IN URINE
7	12/ 2/25	0		0
	12/ 2/25	Cinchophen	105 gr.	0
	12/ 3/25	"		V. F. T.
	12/ 4/25	0	0	0
	12/ 5/25	0	0	0
	12/ 6/25	0	0	0
	12/ 7/25	0	0	0
8	3/24/25	0	0	0
	3/25/25	Cinchophen	135 gr.	–
	3/26/25	"		+
	3/27/25	0	0	0
	3/28/25	Cinchophen		0
	3/29/25	"		0
	3/30/25	"		0.12%
	3/31/25	"		+
	4/ 1/25	"	495 gr.	+
	4/ 2/25	"		0.45%
	4/ 3/25	"		0.25%
	4/ 4/25	"		+
	4/ 5/25	"		0.33%
	4/ 6/25	"		+
	4/ 7/25	"		+
9	4/ 2/25	0	0	0
	4/ 2/25	Cinchophen	140 gr.	0
	4/ 3/25	"		0.25%
	4/ 4/25	0		–
	4/ 5/25	0		–
	4/ 6/25	0		–
	4/ 7/25	0		0.23%
	4/ 8/25	0		0
	4/ 9/25	0		0.20%
	4/10/25	0		0
	4/11/25	0		0
	4/12/25	0		0
	4/13/25	Cinchophen	75 gr.	0
	4/14/25	Tolysin		+
	4/15/25	"		–
	4/16/25	"		0.27%
	4/17/25	"	Amt. undetermined	0
	4/18/25	"		0
	4/19/25	"		0
	4/20/25	"		0
	4/21/25	"		0
	4/22/25	"		0
	4/23/25	"		0.18%
10	5/31/25	0	0	0
	6/ 1/25	Tolysin	825 gr.	+
	6/ 2/25	"		+
	6/ 3/25	0		+
	6/ 4/25	0		+
11	12/ 1/25 to 12/ 8/25	Cinchophen	790 gr.	
	12/ 9/25	0	0	0.547%
	12/10/25	Sod. Sal.		0.20%
	12/11/25	" "	350 gr.	0
	12/12/25	" "		0

TABLE I—CONT'D

CASE NO.	DATE	DRUG	AMOUNT OF DRUG	REDUCING SUBSTANCE IN URINE
12	3/14/25	0	0	0
	3/14/25	Cinchophen	75 gr.	0
	3/15/25	0		0
	3/16/25	Cinchophen		+
	3/17/25	"		0
	3/18/25	"		+
	3/19/25	"		+
	3/20/25	"	405 gr.	+
	3/21/25	"		0.25%
	3/22/25	"		0.18%
	3/23/25	"		0.16%
	3/24/25	"		0.20%
	3/25/25	"		0.31%
	3/26/25	0	0	+
	3/27/25	Cinchophen	30 gr.	+
	3/28/25	"	15 gr.	−
	3/29/25	0		−
	3/30/25	0		0
	3/31/25	0		0
	4/ 1/25	0		0
	4/ 2/25	0		+
	4/ 3/25	0		0
13	12/ 9/25	0	0	0
	12/10/25	Cinchophen	140 gr.	0
	12/11/25	"		0.27%
	12/12/25			0
	12/13/25	Sod. Sal.		0.15%
	12/14/25	" "		0
	12/15/25	" "		0
	12/16/25	" "	740 gr.	V. F. T.
	12/17/25	" "		Less than 0.15%
	12/19/25	" "		0
	12/20/25	" "		Less than 0.15%
	12/21/25			Less than 0.15%
	12/22/25			0
	12/23/25			Less than 0.15%
	12/25/25			Less than 0.15%
	12/26/25			0
	12/27/25			
	12/28/25			
	12/29/25			
	12/30/25			

sionally this leads to incorrect diagnosis, at least temporarily, in a well regulated hospital and may be a more frequent cause of error in the practice of medicine where laboratory methods are not so readily available. In view of the fact that the diabetic patient is not uncommonly afflicted with pains in the legs and elsewhere, and is frequently given salicylates for relief, the occurrence of an additional reducing substance in the urine is very important. These reducing substances are particularly important if a physician is forced to rely on urine sugar as a criterion of the patient's condition.

With these points in mind, urine was collected in twenty-four hour specimens from a series of rheumatic patients before and after they were given sodium salicylate, cinchophen and tolysin. The urine was tested with Benedict's qualitative and quantitative reagents for reducing substances. In all cases fermentation, phenyl hydrazin and polariscopic tests were also made to prove the absence of sugar. In a few instances sugar tolerance tests were also made during the time the reducing substance was present in the urine.

The following tables show that patients receiving cinchophen, sodium salicylate or tolysin invariably have reducing substances in the urine which do not ferment with yeast, give no osazone formation, and rotate the plain of polarized light to the left. The time of their appearance in and disappearance from the urine depends upon dosage and also individual factors which are variable.

The above mentioned drugs themselves, with the exception of tolysin, do not reduce Benedict's solution in vitro, and none gives a positive fermentation test. Another interesting observation was the constant disappearance of these reducing substances from the urine after varying periods of time (twenty-four to eighty-six hours).

Fermentation and phenyl hydrazin tests on the urine, when the reducing substance was present, gave a positive reaction in no instance. The urine invariably rotated the plane of polarized light to the left.

TABLE II

CASE NO.	DATE OF URINE SPECIMEN	REDUCING SUBSTANCE	REDUCING SUBSTANCE IN 24 HR.	REDUCING SUBSTANCE IN 48 HR.	REDUCING SUBSTANCE IN 72 HR.	REDUCING SUBSTANCE IN 86 HR.
1	12/29/25	+	0			
2	12/27/25	+	0			
3	12/28/25	+	0			
6	12/22/25	+	+	–	V. F. T.	0
6	12/27/25	+	+	0		
13	12/27/25	+	0			
Case not reported in Table I	12/20/25	+	+	V. F. T.	0	

Sugar tolerance tests were done on cases 8, 9, and 12, on April 7, January 7, and March 28 with the following results:

CASE 8

Blood sugar.....0.11%
Blood sugar 45 minutes after 50 gm. glucose by mouth.....0.15%
Blood sugar 1½ hours after 50 gm. glucose by mouth.....0.11%

CASE 9

Blood sugar.....0.10%
Blood sugar 45 minutes after 50 gm. glucose by mouth.....0.15%
Blood sugar 1½ hours after 50 gm. glucose by mouth.....0.15%

CASE 12

Blood sugar.....0.10%
Blood sugar ½ hour after 50 gm. glucose by mouth.....0.13%
Blood sugar 1½ hours after 50 gm. glucose by mouth.....0.10%

SUMMARY

1. The purpose of this paper is to recall the fact that cinchophen, salicylates and tolysin give reducing substances in the urine which may be confused with sugar by the copper reduction test alone.

2. Data are given on thirteen cases.

3. These reducing substances do not ferment nor produce osazones but rotate the plane of polarized light to the left.

4. Their presence and quantity in the urine does not depend upon dosage alone.

5. They disappear from specimens of urine in from twenty-four to eighty-six hours.

PROPHYLACTIC AND THERAPEUTIC POSSIBILITIES OF THE TWORT-D'HERELLE'S BACTERIOPHAGE*

(Preliminary Paper)

By Lloyd Arnold, M.D., and Emil Weiss, M.D., Chicago, Ill.

D'HERELLE has applied the term bacteriophagy to the phenomenon which consists essentially in a dissolution or lysis of bacteria through the operation of a principle which he has called bacteriophage. This lytic substance has been used as a prophylactic and a therapeutic agent in some infectious diseases.[1] The usual method of obtaining this bacteriophage, that is to be used for such purposes, is by adding a small amount of the lytic agent to a broth suspension of a young culture of the susceptible bacteria and incubating until clearing of the broth takes place. This cleared or lysed culture is then passed through a Berkefeld candle, and the sterile filtrate is used for prophylactic and therapeutic purposes. During the process of lysis of the young bacteria the active lytic principle is increased in concentration.

We have used another method of preparation of the bacteriophage in this laboratory.[2] A layer of sterile 2 per cent agar in distilled water is poured into a Petri dish; this is covered with a piece of sterile tissue paper, and a layer of nutrient agar is poured on top of this paper. A drop of the lytic agent and a loop of susceptible bacteria are added to the surface of this top layer in the usual manner and smeared well with a sterile bent-glass spreader. After twenty-four hours incubation, the top layer with the irregularly shaped, so-called lysogenic colonies is removed with the adjacent tissue paper. This layer is discarded. The bottom layer is now removed and extracted with distilled water and the extract passed through a Berkefeld filter. Experiments have shown that this layer contains a strong bacteriophage and a minimum content of bacterial proteins.[3] For convenience of description, the broth cleared growths will be referred to as d'Herelle's method and the latter procedure as the bottom-layer extraction method.

It is apparent that either of these filtrates contains dissolved bacterial proteins and the by-products of metabolism of the bacteria that have grown in the culture medium. The culture of bacteria must be actively growing before lysis takes place. We have no evidence of lytic activity taking place in adult bacteria. Lysis reaches its maximum during the period of the life cycle of the bacteria when they are reproducing by geometrical progression. It is interesting in this connection to recall that Levaditi[4] has observed that the vaccine virus is only proliferated in actively growing ectoendodermic cellular elements; he thinks that there must be a "karyokinetic rejuvenescence" as a result of a previous irritation before the virus can be cultivated in such tis-

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sue. The toxins in cultures of B. tetani, B. diphtheria, and such "exotoxin" producing bacteria do not reach their maximum concentration until after several days incubation; in fact, there are several days intervening between the period of growth and that of the highest toxin titer. This is also true for Staphylococcus aureus toxins.[5] The hemolysins produced by some strains of staphylococci are not formed until several days after maximum growth has occurred.[6] The hemolysins produced by streptococci seem to reach their highest titer at the time of maximum growth, although these lysins seem to be nonantigenic.[7, 8]

In the use of bacteriophage for prophylactic and therapeutic purposes it is necessary to distinguish between the effects produced by the bacterial proteins and those produced by the active lytic agent. It is logical to suppose that if only lysis occurred the dissolved bacterial proteins would constitute an ideal antigen. When vaccines are injected for immunization purposes, we think the dead bacteria are dissolved, lysed, or broken up by the ferments that are in the fluids and cells present in the area during the stage of inflammation caused by the presence of the injected bacteria. The bacterial protein liberated in this manner is carried away by the blood and lymph stream and serves as the antigen that causes the production of antibodies. Then if we possessed a lytic agent capable of producing lysis but not accompanied by proteolysis, we should have an ideal antigen.

Several workers[3, 9] have attempted to show that the lysis that occurred as the result of phagic activity was not accompanied by proteolysis. These results are not conclusive; they only show that probably proteolysis does not take place. All observers have noticed that a broth inoculated with a susceptible bacteria and phage grows very rapidly for the first part of the logarithmic phase of its life cycle before lysis begins.[2, 10] Bacterial counts often show the total number of bacteria increased beyond that number present in the control tube without phage at the time just previous to rapid lysis or clearing of the visible bacterial suspension. We have many reasons to think that during the active lytic period there is continuous growth and prompt lysis, etc. These two phenomena are most probably going on at the same time. The rapid clearing of the culture as well as the development of secondary visible and phage-resistant strains in the culture probably depend upon these two processes that are going on at the same time. Arnold and Weiss[3] used the total nonprotein nitrogen, and since then we have used the Van Slyke method of determining amino-nitrogen. Ionescu-Mihaiesti[9] used formol titration. Both workers were unable to demonstrate a marked difference between the lysed culture and the control culture of the same age. We do not know as yet how it is possible to have a proper control that will represent the same number of bacteria that are represented in the unknown lysed culture.

Douglas[11] has shown that short periods (four to twelve hours) of tryptic digestion of acetone-extracted Shiga vaccine causes a change in its antigenic properties. The agglutinating and opsonic antibodies do not appear in the immunized animals' serum. The bactericidal, precipitating, and neutralizing powers are developed as in the control vaccine treated animals. Allison[12]

using "lysozyme" from egg white to dissolve Streptococcus fecalis, found the bacteriolysins and agglutinins absent, the opsonins diminished, but the bactericidal power and the complement-fixing antibodies were the same as in the vaccine-injected controls. We do not wish to discuss the question of the unity of antibodies, but we have shown in former work[3, 13] that there were present in the serum of animals injected with phagic lysed cultures all of the antibodies that could be demonstrated in the control animals injected with dead (vaccine) and living bacteria. This seems to us to be more conclusive evidence than the uncontrollable chemical results already mentioned that proteolysis does not accompany bacteriolysis due to phagic action.

The bacteria dissolved by phage are young growing bacteria. Their protein content should make good antigenic material. Heat, attenuation, and chemical changes due to antiseptics, etc., are avoided. We can conclude then that probably the soluble bacterial proteins in the sterile filtrate of the lysed culture of bacteria are ideal antigens theoretically.

All the studies that have been made with the use of bacteriophage as a prophylactic and a therapeutic agent have had in the injected material these lysed bacterial proteins as well as the transmissible bacteriolytic principle. We have recently published a method of obtaining a bacterial protein-free bacteriophage.[14] Since this work has been published, we have attempted to purify further the bacteriophage; but up to the present we have not succeeded in doing so. We will refer to the usual bacteriophage as "bacterial protein phage" and to the purified bacteriophage as "bacterial protein-free phage."

Bruynoghe and Maisin[15] and Gratia[16] injected d'Herelle's bacteriophage subcutaneously in normal individuals. They describe a febrile reaction with chills, insomnia, etc., persisting for about forty-eight hours. Locally the injections produced erythema and edema which lasted for several days. The reaction was the same if antityphoid or antistaphylococcus bacteriophage were used. The reaction was, in other words, what one would expect to follow any injection of a material containing the amount of foreign proteins present in the bacteriophage they used.

Courcoux, Philibert, and Cordey,[17] Munter and Boenheim,[18] and Zdansky[19] have treated infections of the urinary tract by subcutaneous injections of bacteriophage. Gratia[20] and Gougerat and Peyre[21] have treated staphylococcus skin infections by subcutaneous injections of a staphylococcus-bacteriophage. McKinley[22] treated several different infections with subcutaneous injections of bacteriophage. Beckerich and Handuroy,[23] and d'Herelle[1] have used the subcutaneous injections of bacteriophage in the treatment of typhoid fever. This is by no means a literature review of the various infections treated with subcutaneous injections of bacteriophage but illustrates the variety of diseases that have been so treated by some workers. In reading these reports, the nonspecific or foreign protein reaction is outstanding where a clinical improvement has been reported. The injection of the bacterial protein-free bacteriophage does not cause a change in the distribution of the peripheral leucocytes or a change in the heat regulative mechanism of rabbits. The antibacteriophage content of the serum of animals after bacterial protein-

free bacteriophage has been injected is very high, but there is no evidence of antibacterial bodies in such serum. We wished to study experimentally in the rabbit the relative protective action of bacteriophage, as we are now preparing it, free from antigenic bacterial proteins.

EXPERIMENTS

Time of Appearance of Bacterial Antibodies After Injection of Dead, Living, and Phage-Lysed B. Typhosus

Rabbits were immunized by intravenous injections of bacterial protein bacteriophage (typhoid), B. typhosus vaccine, and living B. typhosus. The vaccine was prepared by heating a twenty-four hour old broth culture to 60° C. for one hour. The living bacteria used were twenty-four hour old broth cultures. The first injection was 0.5 c.c. of each; after seven days 1 c.c., after fourteen days 2 c.c., and after twenty-one days 3 c.c. On the seventh, fourteenth, and twenty-first days just before reinjection, blood was taken to determine the agglutinin and opsonic titer against B. typhosus. Table I gives in condensed form the results of this experiment. The bacteriophage, containing the filtered, lysed B. typhosus, stimulated or caused to appear in the blood agglutinins and opsonins quicker than did the dead and living B. typhosus. The ultimate titer was never so high as was found with the use of the latter two as antigens. This experiment has been repeated using 24 rabbits instead of the 12 recorded in Table I. The results were the same.

TABLE I

AGGLUTININS AND OPSOSINS PRODUCED BY TYPHOID BACTERIOPHAGE, LIVING AND DEAD TYPHOID BACILLI*

ANTIBODIES		ANTIBODIES OF											
		TYPHOID BACTERIOPHAGE				LIVING TYPHOID BACILLI				DEAD TYPHOID BACILLI			
	NO. OF RABBITS	DATE OF TITRATION											
		10/10	10/17	10/24	10/31	10/10	10/17	10/24	10/31	10/10	10/17	10/24	10/31
Agglutination Titer	1	160	1000	2000	4000	20	10000	64000	128000	10	2000	16000	16000
	2	320	1000	3000	4000	10	8000	32000	32000	10	4000	32000	64000
	3	160	1000	6000	8000	20	4000	64000	128000	10	4000	16000	32000
	4	80	1200	5000	6000	40	6000	64000	128000	20	8000	32000	32000
Opsonic Index	1	6.4	9.1	16.3	24.5	4.1	19.5	43.1	76.2	3.3	14.1	26.7	27.6
	2	6.9	8.5	15.1	22.4	3.6	17.6	31.5	33.1	2.8	17.9	31.6	45.3
	3	6.2	9.3	14.1	21.7	3.8	15.8	38.7	62.6	3.9	20.1	24.8	51.5
	4	5.9	8.4	13.6	19.2	4.5	16.6	45.9	81.4	3.6	19.2	32.7	34.8

*All animals were injected upon 10/3, 10/10, 10/17, and 10/24.

We are convinced that these results cannot be attributed to individual variation in the rabbits used by us. The same experiment was carried out with the use of B. dysenteriae Shiga as the antigen. On account of the toxicity of Shiga for rabbits, 0.25 c.c. was injected intravenously as the initial dose. Shiga phage, vaccine, and living bacteria were used. One set of rabbits were immunized with the phage at two day intervals instead of seven day intervals. This was done to see if the interval selected had an influence upon the antibody response of the organism against the lysed bacterial proteins. Twenty-

TABLE II

AGGLUTININS AND OPSONINS PRODUCED BY INJECTION OF SHIGA BACTERIOPHAGE; LIVING, DEAD SHIGA BACILLI, AND SHIGA AUTOLYSATES*

ANTIBODIES	NO. OF RABBITS	SHIGA BACTERIOPHAGE SEVEN-DAY INTERVALS				SHIGA BACTERIOPHAGE TWO-DAY INTERVALS				LIVING SHIGA BACILLI				DEAD SHIGA BACILLI				SHIGA AUTOLYSATES			
		DATE OF TITRATION																			
		10/16	10/23	10/30	11/6	10/16	10/23	10/30	11/6	10/16	10/23	10/30	11/6	10/16	10/23	10/30	11/6	10/16	10/23	10/30	11/6
Agglutination Titer	1	1500	3000	4000	8000	650	2500	7000	14000	400	10000	25000	50000	200	5000	14000	30000	100	2500	12000	24000
	2	1000	2500	4500	9000	1000	3000	8500	16000	250	8000	20000	35000	100	3000	9000	25000	80	4000	8500	20000
	3	600	1800	3200	6500	800	2500	6000	12000	300	9000	30000	75000	350	6000	16000	40000	40	2000	9000	18000
	4	1200	2000	4000	8500	600	2000	4500	10000	400	8000	26000	40000	150	4500	12000	36000	160	3000	10000	25000
Opsonic Index	1	12.1	13.8	16.2	18.5	8.4	14.2	17.4	25.6	4.9	16.7	26.6	48.6	3.2	12.8	22.7	38.0	2.6	13.4	19.8	29.4
	2	8.5	12.3	15.1	16.6	9.3	13.6	18.7	26.1	3.8	15.2	22.9	34.6	2.9	11.9	18.2	35.3	2.1	15.8	18.2	25.7
	3	7.3	14.2	17.5	18.8	9.6	15.7	16.8	22.4	3.4	17.5	28.5	59.8	3.6	16.2	23.5	44.3	1.8	12.6	19.1	24.2
	4	8.9	12.1	15.9	17.5	7.1	11.9	15.5	20.2	4.1	14.3	27.3	42.2	3.1	12.4	21.4	39.5	3.3	13.5	20.3	31.6

*First injection was made on 10/9, others followed at intervals noted on table.

four hour old agar slants of Shiga were each washed off with 5 c.c. of distilled water, placed at room temperature for four days, and passed through a Berkefeld candle. This filtered Shiga autolysate was used as an antigen. The results of this experiment are given in Table II. The bacterial protein phage causes the appearance of antibodies sooner than living, dead, or autolysed Shiga bacilli. The ultimate titer is not so high after phage injection. This experiment was repeated again, using another 20 rabbits. The results did not differ from those recorded in Table II.

Relative Degree of Protection Against Lethal Dose of Homologous Bacteria after Injection of Dead, Living, and Phage-Lysed B. Dysenteriae Shiga

We were interested in the question as to whether a rabbit would be protected against a lethal dose of Shiga bacilli after immunization with the bacterial protein phage to the same extent as would be shown after vaccine, living, or autolysed Shiga. Sixteen rabbits were injected as in the previous experiment—4 with Shiga phage, 4 with dead, 4 with living, and 4 with autolysed Shiga bacilli. The initial intravenous injection was the same. After seven days blood was taken to test for agglutinins, and 1 rabbit of each series with 2 uninoculated controls was given intraperitoneal lethal dose of Shiga bacilli (one-half of a twenty-four hours old agar slant culture). The only rabbit that survived was the 1 that had received the 0.25 c.c. Shiga phage seven days previously. All the other 3 with the 2 controls died between forty-eight and seventy-two hours. The remaining 3 rabbits of each series were reinjected on the seventh day as in the Shiga agglutinin experiment. Seven days after the second injection, 1 rabbit of each series was given 1½ lethal doses of Shiga bacilli. The only one to die was the autolysate rabbit. The other 3 were not killed by the 1½ lethal dose. Seven days after the third injection, 1 rabbit of each series received 2 lethal doses of Shiga bacilli. All survived. Seven days after the fourth injection the remaining rabbit of each series was given 3 lethal doses of Shiga bacilli. All survived. The intravenous injection of the phage with its lysed bacterial protein content, protects the rabbit against a lethal dose of the homologous bacteria sooner than does the living, or dead bacteria, or their autolysates. Topley[24] records a protection in mice against a lethal dose of B. aertrycke when the phage-lysed filtrate is injected intraperitoneally fourteen days before the lethal dose of the homologous bacteria is given.

D'Herelle[1] claims that the larger the dose of the bacteriophage the greater the delay in the immunity. This seems unreasonable, unless the dose is so large that the bacterial protein content is sufficient to cause intoxication and interfere with antibody production. He does not mention this latter reaction as having occurred in the barbone in buffaloes which he used for this work. We injected 3 rabbits with 0.2 c.c. bacterial protein Shiga phage, 3 with 1 c.c., 3 with 2 c.c., and 3 with 4 c.c. of the phage. After seven days 1 from each set was given 3 lethal doses of the living Shiga bacilli. All lived and did not show any signs of a reaction. After fourteen days 1 rabbit from each series was given 6 lethal doses and after twenty-one days the remaining rabbit in each series received 12 lethal doses. All of the animals lived in both experi-

ments. The larger dose did not delay the protection within the period of our experiment.

D'Herelle observed hypersusceptibility to infection of the homologous bacteria when animals were repeatedly injected with bacteriophage. We have never observed this reaction, although over 60 rabbits have been injected with our bacterial protein-free bacteriophages, typhoid, Shiga, and staphylococcus. Rabbits that have been injected with purified bacteriophage contain in the serum a high titer of antibacteriophagic bodies, but they are just as susceptible to the lethal dose of the homologous strain as are the normal uninjected control animals. Where there is a contamination of the phage with the homologous bacterial proteins, protection has always been observed against the lethal dose of the same bacteria.

The Toxicity of Bacteriophage

D'Herelle states that old bacteriophage is not as toxic as freshly prepared phage. We have substantiated this observation. Five cubic centimeters of a freshly prepared typhoid bacteriophage is the lethal dose for a rabbit. The same amount of old phage makes the animal sick for a day, but it always recovers. We have treated freshly prepared and ten months old typhoid bacteriophage with nine parts of 15.55 per cent sodium sulphate solution for two hours at 37° C. The precipitate was collected on filter paper and redissolved in an amount of normal salt solution equal to the original volume of bacteriophage. This solution was then passed through a Berkefeld candle. Five cubic centimeters of this redissolved precipitate, containing the bacterial proteins fraction of the freshly prepared typhoid phage, is as toxic as the same amount of the original material. Five cubic centimeters of the redissolved precipitate of the ten months old phage has the same toxicity as a corresponding amount of old bacteriophage. We conclude that the toxicity manifested upon injection of bacteriophage is due to the bacterial protein and other precipitable material.

It is well known that rabbits are more susceptible to B. dysenteriae Shiga than they are to B. typhosus. We have found that the Shiga phage is not so toxic as typhoid phage. Five cubic centimeters of Shiga phage caused a slight reaction, but after the second day the animals returned to normal. Probably there is only a small amount of exotoxins present in such lysed filtrates, while the bacterial protein and endotoxin content is very high.

Experimental Therapeutic Use of Bacterial Protein-Containing and Bacterial Protein-Free Bacteriophage

We have described in detail our method of the quantitative estimation of bacteriophage.[13] This method consisted, briefly, of determining the smallest amount of bacteriophage added to the surface of an agar plate, seeded with a standard loop of susceptible bacteria, that would cause all the colonies to be irregular or lysogenic in outline. All observers have noted that a large dose of phage would cause inhibition of growth of susceptible bacteria. In this experiment we have titrated the amount of bacteriophage that would inhibit the growth of a given dose of bacteria. After the lethal dose of the

bacteria for the animal has been determined, we can then figure the amount of bacteriophage that should be added to this lethal dose of susceptible bacteria to cause inhibition of growth.

In the following experiments we have injected with the lethal dose of B. typhosus and B. dysenteriae Shiga just the amount of bacteriophage that should inhibit the growth in vitro on agar plates. Twenty rabbits were used in Experiment I, 4 for each series, and 24 rabbits were used in Experiment II.

EXPERIMENT I

A. ½ lethal dose living B. typhosus (1 c.c.) ---------- living
B. 1 lethal dose living B. typhosus (2 c.c.) ---------- died 2-4 days
C. ½ lethal dose living B. typhosus and inhibiting dose protein phage (4 c.c.) ---- died 1-2 days
D. 1 lethal dose living B. typhosus and inhibiting dose protein phage (8 c.c.) ---- 4-12 hours
E. 1 lethal dose living B. typhosus and inhibiting dose protein-free phage (8 c.c.) ---- living

EXPERIMENT II

A. 1 lethal dose living B. Shiga (1 c.c.) ---------- died 3-4 days
B. 1 lethal dose living B. Shiga and inhibiting dose protein phage (8 c.c.) ---------- Reaction 1-2 days, living
C. 1 lethal dose living B. Shiga and ¾ inhibiting dose protein phage (6 c.c.) ---------- Reaction 3-7 days, living
D. 1 lethal dose living B. Shiga and ½ inhibiting dose protein phage (4 c.c.) ---------- Reaction 3-7 days, living
E. 1 lethal dose living B. Shiga and ½ inhibiting dose protein phage (2 c.c.) ---------- died 3-4 days
F. 1 lethal dose living B. Shiga and inhibiting dose protein-free phage (9 c.c.) ---------- living

Experiment I illustrates the toxicity of the bacterial protein typhoid bacteriophage and the immediate protection that is afforded by injection of the bacterial protein-free nontoxic phage. Where the phage with its lysed bacterial protein content was injected, death occurred sooner than in the controls. Series *A* compared with Series *C* brings this out clearly. Neither injected alone were lethal, but combined there appeared to be a summation of the toxicity of the 2 substances. Experiment II illustrates practically the same thing with B. dysenteriae Shiga. The effect of fractional amounts of the inhibiting dose of the bacterial protein phage is apparent. As mentioned before, the Shiga phage was not so toxic as the typhoid phage. This is shown in the experiment. The nontoxic purified bacteriophage shows immediate protection as in the previous experiment.

Most of the animal experimental work done by d'Herelle was with rabbits, guinea pigs, and mice, using Shiga bacteriophage. This phage does not have the same toxicity as a true "endotoxin" producing bacteria such as B. typhosus. The content of the phagic suspension is not so toxic, and the bacilli lysed in vivo do not lead to an intoxication comparable to the same number of B. typhosus.

Five minutes after injection, in Experiment II, blood was taken from the ear vein of the opposite side of 2 rabbits from each series. Ten drops were put directly upon the surface of an agar plate and smeared well with a bent glass spreader. After twenty-four hours incubation, *A* had a heavy growth of regular shaped colonies; *B* was practically sterile, only 3 or 4 very irregular shaped colonies; *C* and *D* showed better growth, but the majority of the colonies were "moth eaten" or irregular in shape; *E* had a good growth

with relatively few colonies of the lysogenic type; *F* was the same as *B* plate. Within the limits of our experiment, the in vivo lysogenic activity of the bacteriophage and susceptible bacteria were roughly proportioned to the in vitro inhibitory activity.

TABLE III

PHAGOCYTIC INDEX AFTER INTRAVENOUS INJECTION OF B. DYSENTERIAE SHIGA AND BACTERIOPHAGE

(Experiment II)

NO. OF RABBITS	MATERIAL INJECTED	WHITE BLOOD COUNT AFTER INJECTION					PHAGOCYTIC INDEX AFTER INJECTION				
		BEFORE	1	3	6	12	24	1	3	6	12	24
		HOURS						HOURS				
1	1 Lethal dose of Bacilli Shiga	6700	16300	5900	6100	6700	6200	0.9	0.82	0.9	0.75	0.88
2	1 Letha Dose of Bacilli Shiga	7150	19250	7100	7600	8150	8400	0.76	0.6	0.8	0.9	0.7
3	1 Lethal Dose Corresponding Amount of + Routine Phage	8200	15100	10200	8900	9100	7800	1.6	1.1	0.5	0.65	0.71
4	1 Letha Dose of Bacilli Shiga	9200	18700	9450	8300	6900	7500	1.3	1.05	0.9	0.91	0.84
5	1 Lethal Dose + ¾	8300	16900	8400	9100	7600	8200	1.02	0.95	0.89	0.92	0.85
6	1 Letha Dose of Bacilli Shiga	8700	19600	7650	8350	6700	9400	1.15	0.98	0.93	0.86	0.78
7	1 Lethal Dose + ½	8100	14500	10200	9300	9100	8600	1.08	0.91	0.83	0.76	0.82
8	1 Letha Dose of Bacilli Shiga	7300	15200	9800	8100	8800	7700	1.03	0.99	0.94	0.8	0.91
9	1 Lethal Dose + ¼	6200	13400	7500	7100	8500	8100	1.09	1.01	0.89	0.86	0.74
10	1 Lethal Dose of Bacilli Shiga	7850	16300	9200	8650	8900	8700	1.05	0.94	0.92	0.85	0.71

EXPERIMENT III

A. Lethal dose of B. dysenteriae Shiga ---------- died 3-4 days
B. Lethal dose of B. dysenteriae Shiga and inhibiting dose of bacterial protein phage, given immediately ---------- slight reaction, living
C. Lethal dose of B. dysenteriae Shiga and inhibiting dose of bacterial protein phage, given after twenty-four hours -- reaction lasting 4-7 days, living
D. Lethal dose of B. dysenteriae Shiga and inhibiting dose of bacterial protein phage, given after three days ---------- One died immediately after phage injection, one on the fourth and one on the fifth day after Shiga injection.

Bacteriolysis in vitro cannot be compared with the same phenomenon in vivo. There is no conclusive evidence that there is bacteriophagic lysis in vivo. These and other similar experiments show that the lytic agent must be administered in sufficient quantity to exercise a growth inhibitory effect upon the bacteria. The phagocytic index was determined from the blood from the same rabbits. The results are recorded in Table III. We have substantiated d'Herelle's observation that his bacteriophage causes an increase in the phagocytic power of leucocytes.[1, 3] We did this experiment to find out what part phagocytosis might play in the above reaction. We think we are justified in concluding that phagocytosis does not play an important rôle in the reaction; certainly it only plays a secondary rôle.

We did Experiment III to determine the therapeutic value of the bacteriophage administered at different intervals after the injection of the lethal

dose of the Shiga bacilli. Three rabbits were used for each series, 12 rabbits in all. We have repeated this experiment on 12 more rabbits varying the time of phage injection from eighteen to thirty hours after the lethal dose of Shiga bacilli. In most instances after the onset of paralysis of the extremities, the phage injection leads to recovery. As has been found with antitoxins, when retrogressive changes in certain tissues are extensive, the restoration to the normal does not take place. The experimental therapeutic administration of phage in Shiga infection in the rabbit substantiates d'Herelle's observation. We wish to mention that Shiga phage is one of the most nontoxic bacteriophages we have worked with. It does not compare in toxicity with typhoid phage. For experimental therapeutic use of this phage we have had to free it of bacterial proteins and other toxic substances.

DISCUSSION

All of the antibodies that can be demonstrated in the blood of an immunized animal after the use of B. typhosus, living, dead, and autolyzed can be found after immunization with phage-lysed filtrates of B. typhosus. In addition, we find the antibody titer develops more rapidly and the protection against a lethal dose of the homologous bacteria is manifested more quickly after a single injection of the phage-lysed B. typhosus than after a similar injection of living, vaccine, or autolysates of the same bacteria, although the ultimate antibody titer is not so high. All available evidence seems to indicate that phagie-bacteriolysis is not accompanied with proteolysis.

The degree of protection against lethal doses of the homologous bacteria as a result of a single injection of phage-lysed B. typhosus, B. dysenteriae Shiga, and Staphylococcus aureus is out of proportion to the demonstrable antibody titer in the blood serum at the time that this immunity is enjoyed by the animal.

When phage-lysed filtrates are used for curative or specific therapeutic treatment, the toxicity of the particular protein must be taken into consideration. If the injection of the bacterial protein is accompanied by evidences of intoxication, these proteins should be removed by some of the methods described by us in a former publication.[13] Our results so far indicate that the beneficial effects obtained by phage injections following the administration of lethal doses of the homologous bacteria in rabbits are due to the growth inhibitory effect of the bacteriophage. If smaller doses of phage are administered, these do not protect the animal against the lethal effect of the bacterial infection. In the test tube much smaller doses than these lead to bacteriolysis with ultimate depression of the bacterial growth. This is due to lysis that occurs during the period of maximum growth in vitro. Large doses of bacteriophage in vitro lead to inhibition of growth. In vivo we have found that this dose was necessary to save the animal after administration of a lethal dose of the homologous bacteria. We have been led to believe that the bacteriophage does not increase in *in vivo* as it does in *in vitro* experiments. At least this has not been observed under the conditions of our experiment.

The transmissible bacterial lysins (bacteriophage), freed from toxic lysed

bacterial proteins and administered in sufficient quantity, may offer a curative or therapeutic aid in certain infectious diseases.

In considering bacteriophage from a clinical standpoint, one must separate the bacterial proteins from the active transmissible lytic agent (phage). In all of the work so far recorded, both factors were considered together. Since we have succeeded in separating these two components, we can now determine their respective therapeutic and preventive properties. From our experimental work we think the bacterial protein content of the bacteriophage used heretofore has been the active agent in the improvement in the cases so far recorded. It would be impossible to inject a dose of bacteriophage large enough to cause inhibition of B. typhosus in vivo in typhoid fever because of the toxicity of the accompanying bacterial proteins.

We have not mentioned the administration of bacteriophage per os. There is an entirely different mechanism involved in this reaction. Considerable more work must be done upon the bacteriology and physiology of the upper end of the gastro-intestinal tract[25] before we can hope to utilize per os administration of bacteriophage intelligently.

SUMMARY

The lysed, soluble bacterial proteins in the bacteriophage are antigenic and confer an early active immunity.

Bacteriophage, free of antigenic bacterial proteins, prevents death when injected in the rabbit after a lethal dose of bacteria has been given.

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THE ELECTIVE LOCALIZATION OF BACTERIA IN HEART AND VASCULAR DISEASE*

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THE reproduction of a patient's lesion in animals by the injection of bacteria recovered from a septic focus is most valuable proof of a causal relation of the focus to the systemic disease. Such proof depends upon the fact that bacteria in foci of chronic infection may acquire certain properties which determine their localization on introduction into the experimental animal. This fact was first pointed out and has been repeatedly emphasized by Rosenow.[1] I have studied the elective affinity of bacteria isolated from chronic foci in patients suffering from metastatic infections of the eye,[2] kidney infections,[3] and peptic ulcer,[4] and reported experimental results confirmatory of Rosenow's theory. Recently I have been interested in determining the results of the inoculation of bacteria, principally streptococci, from a series of patients suffering from metastatic heart and vascular disease.

It has long been recognized that endocarditis is of bacterial origin, although a great variety of organisms may give rise to vegetations on the valves. Likewise it is now known that disease of the myocardium is largely the result of the activity of bacteria or their toxins. Numerous investigators have produced endocarditis experimentally. Rosenow[5] has studied the problem of the specific affinity of certain organisms for the heart valves. He found that 84 per cent of the rabbits inoculated with streptococci isolated from foci in patients suffering from lesions of the heart valves had endocarditis at autopsy. Only 14 per cent of the rabbits injected with strains from other sources had similar lesions. Detwiler and Robinson[6] found also that streptococci recovered by blood culture in patients with active endocarditis involved the valves of a high percentage of the rabbits injected. Thalhimer and Rothschild[7] studied the myocardial lesions found in rabbits

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inoculated with streptococci from various sources. In most cases no gross lesions were found. Henrici[8] produced both valvular and myocardial lesions in rabbits with different types of streptococci.

METHOD

All inoculations in my series have been made with organisms, usually streptococci, from dental infection. The method of obtaining the culture material, making the culture, and other technical details have been described fully elsewhere.[2] Rabbits have been injected intravenously with the original broth cultures. The animals averaged about 1500 gm. Each animal was given 5 c.c. of the broth suspension. The animals were killed in three to five days after injection and all organs carefully examined for lesions. No lesion was considered present unless plainly visible to the naked eye of at least two observers.

EXPERIMENTAL OBSERVATIONS

Forty rabbits were inoculated intravenously with the cultures from 10 patients suffering with heart or vascular disease of bacterial origin. At least 2 rabbits were injected from each patient. Eighty-two per cent of animals injected had, at autopsy, some gross heart lesion; 63 per cent showed involvement of the endocardium; 50 per cent had gross myocardial disease (Table I). During the period covered by these experiments 1210 other rabbits were injected with cultures from dental foci in patients not known to be suffering from heart or vascular disease. Of these only 17 per cent had vegetations or hemorrhage of the endocardium; only 9 per cent showed myocardial involvement. Table I shows also the percentage of animals having lesions in other organs. The figures other than those for the heart are much the same in the 2 groups.

TABLE I

LOCALIZATION OF BACTERIA FROM INFECTED TEETH IN HEART AND VASCULAR DISEASE

GROUP	NUMBER OF ANIMALS	NUMBER OF PATIENTS	PERCENTAGE OF ANIMALS SHOWING LESIONS IN							
			JOINT	KIDNEY	MUSCLE	ENDOCARDIUM	MYOCARDIUM	BRAIN	EYE	STOMACH AND DUODENUM
I*	1210	405	60	32	22	17	9	5	14	14
II†	40	10	60	25	22	63	50	2	8	14

*Group I.—Animals inoculated with dental cultures from patients not known to be suffering from heart or vascular disease.

†Group II.—Animals inoculated with cultures from teeth of patients suffering from heart or vascular disease.

The valvular lesions were almost entirely vegetations, often of large size. Occasionally they were large enough to almost completely occlude the valve opening. In some animals only hemorrhages at the base of the valves were found. In 24 animals a record was kept of the valves involved; 17 animals had involvement of the tricuspid valve; 7, the mitral valve. The myocardial involvement consisted often of gross hemorrhage in the heart muscle. In about an equal number of cases the lesion consisted of focal necrosis, usually multiple. These appeared as short, white streaks best seen

when fresh. They are similar to the focal lesions seen in the voluntary muscles.

The case histories and protocols of the animal experiments are given in detail later.

SUMMARY AND CONCLUSIONS

Forty rabbits were inoculated intravenously with bacteria from the infected teeth of a few patients suffering from heart or vascular disease. Eighty-two per cent of the animals showed some heart lesion; 63 per cent had valvular disease, and 50 per cent showed myocardial involvement.

During the same period 1210 rabbits were injected similarly with cultures from patients not known to have heart or vascular disease. Twenty-two per cent of these had some heart involvement, 17 per cent had valvular lesions, and 9 per cent, myocardial disease.

These results are confirmatory proof of Rosenow's theory of elective localization. They emphasize, also, the possible relation of the dental infection to the heart disease.

CASE REPORTS

Acute Myocarditis

CASE 1. *History.*—D. H., a medical student, aged twenty-four years, stated that he had had several acute attacks of rapid heart beginning at the age of twelve years. There was no history of coincident infection at the onset. At the age of eighteen an attack occurred with an abscessed tooth. In May, 1925, he had an attack lasting several hours during which electrocardiograms were taken. These showed the tachycardia to be of a ventricular type (Fig. 2 *A, B, C,*). For several weeks before this attack he had had an infection around a partially erupted third molar tooth. A second electrocardiogram taken in June, 1925, showed a normal heart rate but evidence of myocardial disease (Fig. 2 *D, E, F,*).

Animal inoculations.—At this time the third molar tooth was removed, revealing a pocket of pus from which a pure culture of a streptococcus was obtained. Two rabbits were injected with this culture. One died forty-eight hours later. At autopsy there were numerous areas of hemorrhage in the heart muscle (Fig. 3 *C,* and *D,*). The other animal was killed. This also had a smaller number of hemorrhages in the heart muscle.

Subsequent course.—In October, 1925, the patient had another attack during which he died. At autopsy the heart showed no gross lesions. Sections, however, showed areas of acute infection in the heart muscle (Fig. 3 *A* and *B*).

Acute Auricular Fibrillation

CASE 2. *History.*—H. B. D., aged sixty-five years, a banker, had been having attacks of acute auricular fibrillation for only a short period of time. He had otherwise been in excellent health. The general physical examination was negative except for the heart condition. He had had some indefinite gastric symptoms. There was no hypertension. The dental radiographs showed 3 pulpless teeth only 1 of which showed radiographic evidence of infection. All 3 teeth were extracted. Only 2, the upper right and left second bicuspid, were cultured. Both showed a profuse growth of streptococci.

Animal inoculations.—Two animals were injected with 5 c.c. each of the broth culture of the streptococcus recovered from the upper right second bicuspid. One animal was killed five days later. The examination showed a large vegetation on the tricuspid valve (Fig. 4 *B*) and hemorrhages in the myocardium. There were also hemorrhages in the first part of the duodenum, a few cortical kidney abscesses, purulent fluid in the large joints and some areas of necrosis in the muscle. The second rabbit was killed six days after injection. There were vegetations on the tricuspid valve and in the right auricle (Fig. 4 *C*). The joints showed very slight involvement. There was 1 small abscess in the medulla of 1 kidney and some necrosis in the muscle.

Two rabbits were also injected with the diplococcus recovered from the upper left second bicuspid (Fig. 4 *A*). One showed hemorrhages in the papillary muscle of the left ventricle (Fig. 4 *D*), vegetations and hemorrhages in the endocardium of the right auricle, and some infection around the joints. The other animal showed vegetations on the heart valves and some small focal lesions in the myocardium. There were also a few lesions in the kidney medulla and involvement of the joints and muscles.

Fig. 1.—*A*, culture from periapical dental infection in glucose-brain agar. Note the uniform growth of colonies throughout tube; *B*, culture similarly made in which there is no growth at the top of tube; *C*, photomicrograph of characteristic diplococcus from dental infection.

Endocarditis and Auricular Fibrillation

CASE 3. *History.*—L. C. H., a widow, aged sixty years, working as a clerk, complained of heart trouble. She had had chorea first at twelve years, with recurrent attacks for several years. At fourteen years, she had diphtheria and at twenty-three, scarlet fever. Eight years before, she had had scleritis. For several years she had had albumen and pus in the urine. At one time, removal of a kidney was considered on account of the pyuria.

The patient stated she had been well up to 1912, eleven years before admission, when

she had a severe attack of influenza. Two weeks later, she began to have arthritis, which persisted for six months. She was then well for several months, after which she began to have attacks of rapid and irregular heart. She had to give up work for seven weeks at this time on account of heart symptoms. About once a year, since this initial attack, she had had an attack of heart trouble, incapacitating her for work for from six weeks to four months. During the past year the attacks had been occurring every few days, lasting a few days at a time. The symptoms were worse on exertion. At times the ankles were swollen. Recently the patient had been to the Mayo Clinic where a diagnosis of par-

Fig. 2.—*A*, *B*, and *C*, electrocardiograms made from patient (Case 1) showing a tachycardia of ventricular origin; *D*, *E*, and *F*, electrocardiograms taken one month later. Note the inverted T-wave and change in QRS complex indicative of myocardial disease.

oxysmal auricular fibrillation was made. On admission there was a definite aortic insufficiency without demonstrable cardiac enlargment. The heart rate was slow and regular except for an occasional extra systole. The blood pressure was: systolic, 140; diastolic, 70; The urine showed a few pus cells in clumps. There were 11 pulpless teeth, only 4 of which showed definite roentgenographic evidence of infection.

Animal inoculations.—The lower right second bicuspid and first and second molars were extracted first. All showed a profuse growth of nonhemolytic streptococci. Two rabbits were injected. One had, at necropsy, a few endocardial vegetations, a few abscesses in the

medulla of the kidney and a small amount of purulent fluid in the joint. The other rabbit showed a massive vegetative endocarditis of the tricuspid valve (Fig. 5 *A*), a few lesions in the myocardium, and slight involvement of the joints. One rabbit was injected with the cultures from the lower left bicuspid and second molar. At necropsy, a few vegetations on the heart valves, numerous small abscesses in the wall of the left ventricle, a purulent arthritis, and a few kidney abscesses were found. Two rabbits were injected with the cul-

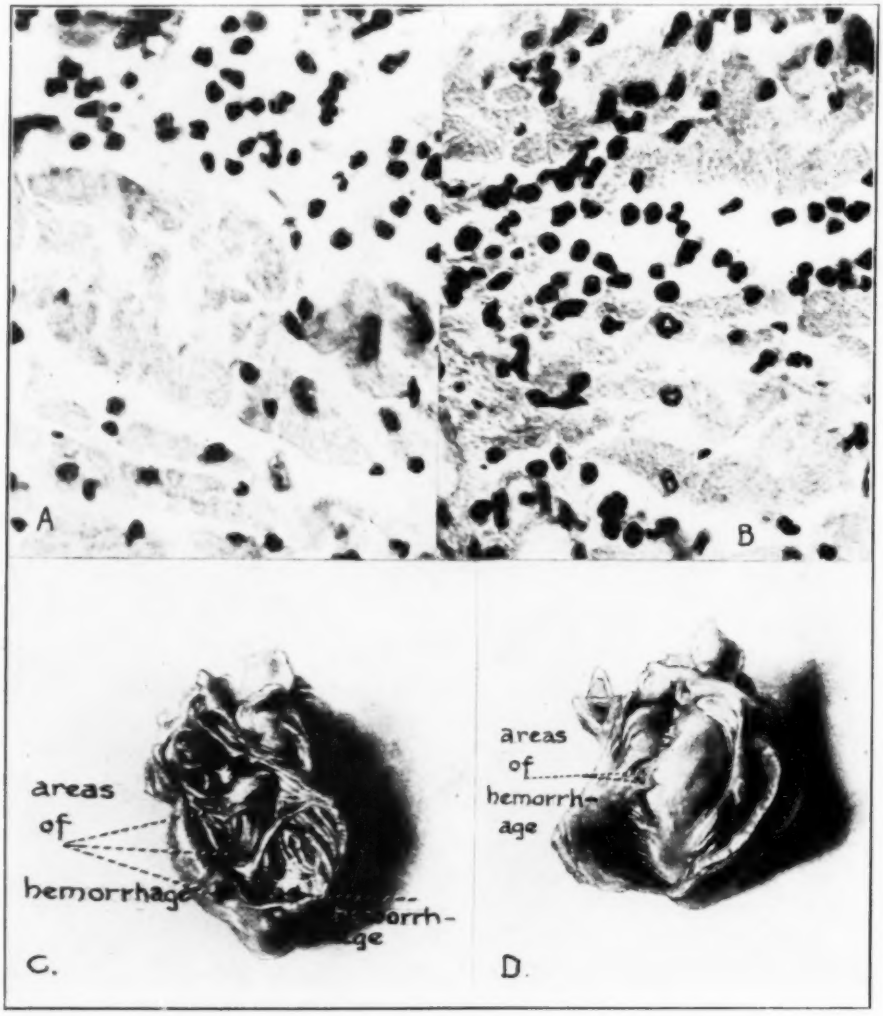

Fig. 3.—*A*, and *B*, sections of heart muscle obtained at autopsy (Case 1). Note the areas of cellular infiltration. Many of the cells are polymorphonuclear leucocytes; *C* and *D*, photographs of hearts of rabbits injected with streptococcus from infected third molar tooth. Note the areas of hemorrhage.

tures from the remaining teeth. One was dead the following day. There were many hemorrhages in the endocardium of the left ventricle and at the base of the papillary muscles. There were also a few hemorrhages and small vegetations in the right auricle near the ventricle. The other rabbit died two days after injection. At necropsy, only early vegetations on the mitral and the tricuspid valves, and mural thrombi in the right auricle were found.

Acute Phlebitis and Myocarditis

Case 4. *History.*—J. W. P., a physician, aged sixty years, had had a phlebitis of the left femoral vein in 1904 following an acute alveolar abscess. Following this there was frequent flare-ups of the dental infection without further signs of systemic disease. In 1914 he began to have anginal attacks which continued to 1916. The attacks were entirely relieved by the removal of an infected tooth. In March, 1923, the root of the bicuspid tooth became infected, and following this he had a recurrence of the phlebitis and anginal attacks. In June, 1923, nonhemolytic streptococcus was recovered from the blood. He became progressively worse, developed myocardial insufficiency and died. At autopsy there were multiple infarcts in the heart muscle.

Animal inoculation.—After the extraction of the bicuspid root, the infection of which had initiated the present illness, cultures were made from the socket and 2 rabbits were injected. The culture showed only a green producing streptococcus. The rabbits at autopsy showed only endocardial vegetations and infarcts of the myocardium. The upper right second and third molars were extracted in July, 1923. A profuse growth of streptococci was obtained from both. Two rabbits were injected. One was dead the following morn-

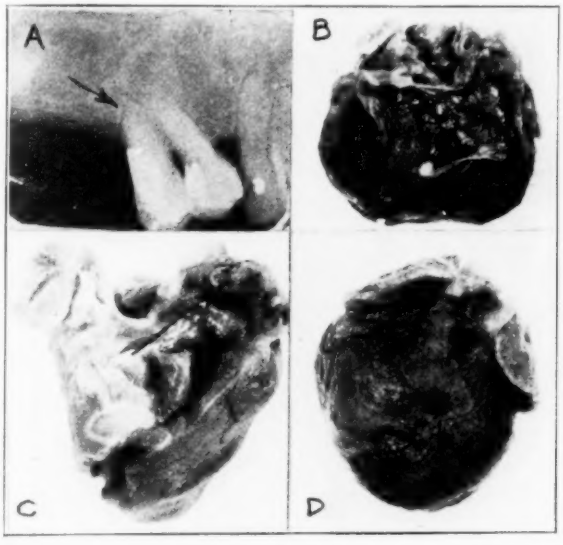

Fig. 4.—*A*, x-ray negative pulpless tooth of patient (Case 2) which showed a profuse growth of streptococci on culture; *B*, vegetations on tricuspid valve of rabbit injected with culture; *C*, heart of another rabbit similarly injected showing multiple vegetations and hemorrhages in the wall of its auricle; *D*, hemorrhage in myocardium.

ing. The autopsy revealed only multiple hemorrhages at the base of the valves. The second rabbit was dead forty-eight hours after injection. The examination showed only vegetations of the heart valve (Fig. 5 *B*).

Thromboangitis Obliterans

Case 5. *History.*—J. C. W., a laborer, aged forty-six years, complained of a painful great toe. For sixteen years he had suffered from pain in the calf of the leg on walking. For two years he had been able to walk only a short distance without resting. For one year he had had trouble in the great toe which he ascribed to an infected toe nail. Part of the toe had been amputated. On examination, the end of the toe at the site of amputation was gangrenous. No pulse could be detected in the anterior or posterior fibial or the dorsalis pedis artery of either foot. The blood pressure was 130/80. The general examination was negative except for infected tonsils and extreme oral sepsis. The urine examination was negative. The hemoglobin was 90 per cent, the white count 12,100. The Wassermann was

negative. The blood chemical examination showed no deviation from the normal. The toe was first amputated, followed later by amputation of the foot.

Animal inoculations.—Several teeth were extracted November 16, 1922. Two rabbits were injected. One showed only a meningitis. The other had at autopsy very large vegetations on the aortic valves (Fig. 6). There were also lesions in the stomach, joints, and kidney.

Myocarditis

Case 6. *History.*—A. L. S., a woman, aged fifty years, had been suffering from a myocarditis with a persistently rapid heart. The dental radiographs showed 4 pulpless teeth which were negative for infection. The cultures showed little growth in agar. From all broth cultures a streptococcus was obtained.

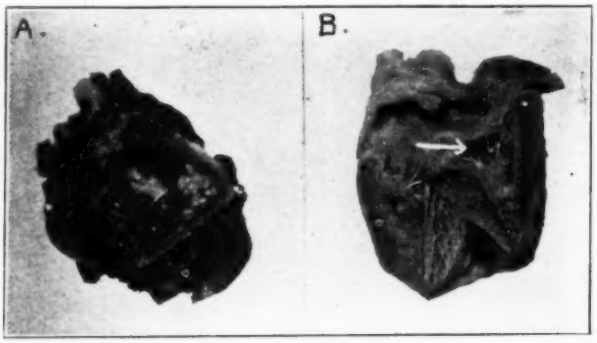

Fig. 5.—*A*, heart of rabbit injected with the culture from teeth of patient with aortic insufficiency and paroxysmal auricular fibrillation (Case 3). Note the large vegetations. *B*, heart of rabbit injected with the cultures from patient with myocarditis (Case 4).

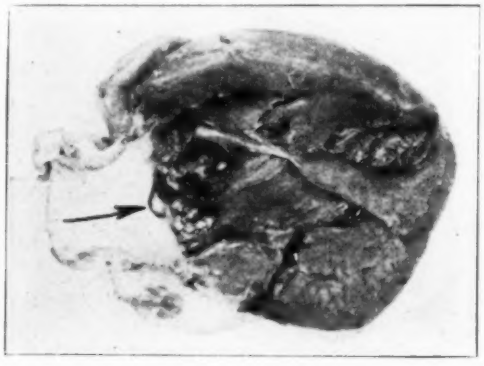

Fig. 6.—Large vegetations on the aortic valves of heart of rabbit injected with cultures from patient (Case 5).

Animal inoculations.—Four rabbits were injected intravenously; 1 animal died in a short while after inoculation; and 1 showed at autopsy, four days after the injection, a marked necrosis of the myocardium of the right ventricle; a second animal showed numerous hemorrhages in the heart muscle; and the third showed hemorrhages in the myocardium and vegetations in the tricuspid valve. These three animals showed some joint involvement; one had also a few lesions in the kidney medulla and in the muscles.

Acute Auricular Fibrillation

Case 7. *History.*—J. M. R., a live stock dealer, aged fifty-eight years, complained of palpitation of the heart. For a number of years he had suffered from chronic arthritis for which the tonsils had been removed, some teeth extracted, and the nasal sinuses drained.

There was now quite marked deformity of the joints but no evidence of active infection. For four or five years he had had attacks of rapid heart lasting only a short while. The present attack had begun the day before. There was moderate dyspnea on exertion. On examination the heart rate was 135-140 and totally irregular. The fluoroscopic examination showed marked dilation of the right side of the heart. There were no signs of valvular disease. The blood pressure was normal. The urine showed only a trace of albumen. Following the removal of the dental infection the heart rate returned to normal and has continued so for two years.

Animal inoculations.—The dental radiographs showed 2 roots (Fig. 7 *A*) which were removed. The cultures showed a profuse growth of streptococci (Fig. 7 *B*). Four rabbits were injected. Three animals at autopsy had hemorrhages in the myocardium. Two had vegetations in the valves. Two had also joint lesions, one an iritis, and one hemorrhages in the stomach and duodenum.

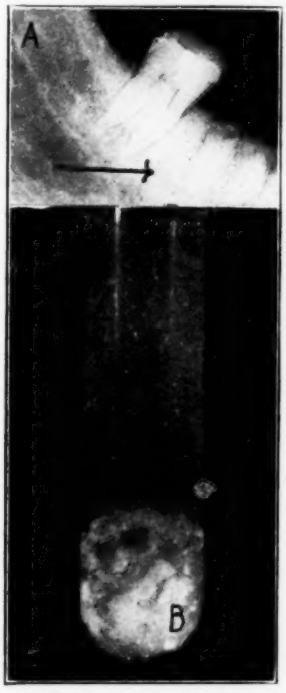

Fig. 7.—*A*, radiograph of alveolar process showing two root tips remaining (Case 7). *B*, culture from root tips. With this organism marked lesions in the heart of animals were produced.

Myocarditis with Angina Pectoris

CASE 8. *History.*—L. B. N., a carpenter, aged forty-two years, complained of recurrent attacks of pain over the heart. The attacks had begun eight months previously, were always brought on by exertion, and were relieved by rest. During one attack the left arm felt numb. He had always been well before the present illness. On examination numerous extra systoles were noted. The blood pressure was 100/75. There was no anemia. The blood Wassermann was negative. The urine showed no albumen, sugar or casts. There were numerous pulpless teeth. The tonsils were large and red. The fluoroscopic examination showed the heart and the aorta of normal size. The tonsils were removed and the pulpless teeth extracted. After the extraction of the teeth the patient felt so much better that he wished to return to work. Two weeks later he began to have dyspnea which increased in severity. He complained of a feeling of pressure in the chest, and developed a pericardial friction rub. Signs of myocardial insufficiency soon developed followed by death about one year after the onset of symptoms.

Animal inoculations.—Two rabbits injected with the broth culture from the tonsils showed no lesions of any kind. Three x-ray positive teeth, the left lower first and second bicuspids, the first molar were first extracted. All showed a profuse growth of streptococci. Two rabbits were injected and killed three days after inoculation. One showed only an arthritis with a pyelonephritis. The other showed a marked necrosis of the myocardium involving almost the entire right ventricle (Fig. 8). A slight joint involvement was the only other lesion present.

One week later 4 more teeth were extracted. The left upper first bicuspid and lateral incisor were x-ray positive and showed a profuse growth of streptococci on culture. The left upper was negative in the radiograph and showed also a profuse growth of streptococci. The left upper cuspid showed only a few colonies in the agar tube and was negative in the

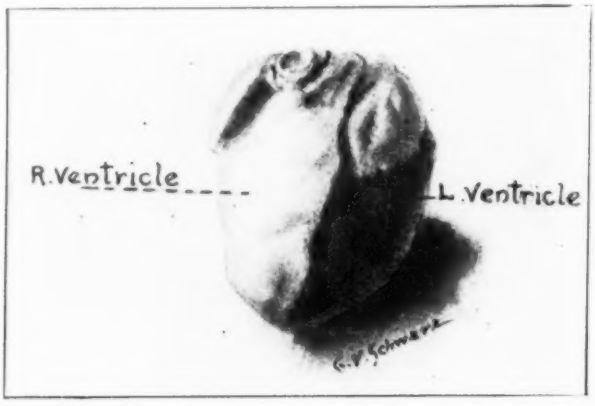

Fig. 8.—Massive necrosis of almost entire right ventricle of rabbit injected with a culture from the infected teeth of a patient suffering from myocardial disease (Case 8).

Fig. 9.—Multiple areas of necrosis in heart muscle and vegetation on the valve of a rabbit injected with the dental culture from a patient (Case 9).

radiograph. Two rabbits were inoculated with the mixed cultures. One showed at autopsy slight joint lesions and yellow plaques in the arch of the aorta. The other showed lesions in the joints and muscles, marked necrosis of the heart muscle, and several vegetations on the mitral valve.

Recurrent Acute Phlebitis and Arteritis

CASE 9. *History.*—C. C. W., a telephone clerk, aged thirty-five years, complained of painful nodules in the leg. The first nodule had appeared at the age of twenty years and remained only a short while. He had had no further symptoms until the age of thirty when he had an attack of influenza. Four months after the attack a red, painful swelling appeared over the right popliteal vein. Following this there were many such swellings over

the veins of both lower extremities. Two years before, the right foot had become swollen and painful. One toe became gangrenous. A few months later the right leg was amputated.

On examination there was a small nodule above the internal condyle on one leg. The general examination was negative. The tonsils were small and deeply imbedded. The blood pressure was 96/58. The urine examination was negative. The white blood count was 8500, and the hemoglobin 85 per cent. The Wassermann test was negative.

Animal inoculations.—The dental radiographs showed 6 pulpless teeth which were extracted. Four rabbits were injected with the cultures. Two showed at autopsy many

Fig. 10.—*A*, area of edentulous bone with a residual infection (Case 10); *B*, culture from curetting of area shown by arrow in *A*. Note the profuse growth of bacteria, especially in the bottom of the tube.

areas of necrosis and hemorrhage in the myocardium and vegetations on the valves, (Fig. 9). One had also areas of necrosis in the voluntary muscles, and both showed hemorrhages in the duodenum. One of the other rabbits showed no lesions, the other only muscle involvement.

Acute Arteritis

CASE 10. *History.*—W. M. B., aged fifty-five years, had had a number of serious infections of probable focal origin. Twenty years before he had had bilateral uveitis with complete loss of vision. At the same time a diagnosis of diabetes mellitus was made. In the following years he had suffered from chronic arthritis and a duodenal ulcer. On examination he was found to have a high grade secondary anemia, a marked arterial hypertension, and the urinary findings of chronic nephritis. Recently he had suffered from a swelling of

the leg and the foot. The swelling was limited to the lower half of the leg and the foot. There was no pulsation in the dorsalis pedis arteries. The condition seemed due to localized arterial disease. Gangrene seemed imminent.

Animal inoculations.—The systemic diseases from which the patient had suffered were recognized as of focal origin but no definite foci had been found. Twenty years before all teeth had been extracted for extensive pyorrhea. The radiograph of the jaws shows numerous areas of apparent infection in the bone. It seemed quite evident that the anatomic changes in the patient were so extensive that removal of foci could be of little value. One suspicous area (Fig. 10 *A*) was exposed and curetted. The bone was quite soft. The culture showed a profuse growth of streptococci which would not grow to the top of the brain broth agar tube (Fig. 10 *B*).

Two rabbits were injected with the culture. Both animals developed a mild iritis. One at autopsy showed no other lesions. The other showed endocardial vegetations, abscesses in the kidney and marked joint involvement.

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THE SUGAR AND CHLORIDE CONTENT OF THE CEREBROSPINAL FLUID WITH SPECIAL REFERENCE TO NEUROSYPHILIS

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THE reducing substance in the cerebrospinal fluid in man has received a certain amount of attention by chemists and clinicians in the last two decades. It has been shown to be essentially a monosaccharid, probably glucose. The normal amount has been variously estimated by different authors, from a minimum of 40 mg. for each 100 c.c. to a maximum of 134 mg. In contrast to this discrepancy Mestrezat, who has done considerable work on the sugar of the cerebrospinal fluid which he believes is allied to if not identical with the true and dialyzable sugar of the blood plasma, gives the normal content as from 55 to 65 mg. for each 100 c.c., with an average of from 59 to 60 mg. Most authors grant a much greater range of normal variation. Thalhimer and Updegraff, by means of the Folin and Wu method, estimated the upper limit of normal at from 60 to 65 mg. Kelley pooled normal fluids and obtained 55 mg. by the same method. The variation in results is probably attributable to difference in method of estimating sugar and precipitating the blood protein. The herculean task of comparing a score or more of reported procedures would be necessary to determine this. A few authors have made parallel determinations by two or three methods, with some interesting observations. For instance, Stevenson found the discrepancy between values obtained by Folin's and Shaffer's methods was greater in patients with encephalitis than in normal persons. He considered this probably due to substances capable of reducing Folin's but not Shaffer's reagent.

The amount of reducing substance has been found to be abnormal in cases of acute and chronic meningitis, encephalitis and diabetes. A moderate amount of work has been done on the cerebrospinal fluid in cases of neurosyphilis. In cases of undifferentiated neurosyphilis increased values were found by Boyd, Verain and Vernet, Kaplan, and Csáki; normal values were reported by Rieger and Solomon, Kahler, and Kraus and Corneille; and decreased values were found by Borberg, Holzmann, Kelley, and Martin. In cases of tabes dorsalis increased amounts were noted by Löwy, Kaplan, Csáki, and Polonowski and Duhot; normal values were found by Kahler, and Rieger and Solomon, and decreased amounts were reported by Borberg and Holzmann. In cases of general paresis high values were found by Csáki, normal values by Briand, Marcel and Rouquier, and Rieger and Solomon, and decreased values by Borberg, Holzmann, Kelley, and Polonowski and Duhot. Alpers, Campbell and Prentiss found an average of 65 mg. in cases of paresis without treatment, and 55 mg. in cases in which treatment had been given; Weston found an average of 71.8 mg. in the cases without treatment and

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72.5 mg. in the cases with treatment. Thus it is seen that there is no unanimity of opinion, but each individual author's findings are essentially the same for the three phases of neurosyphilis. Hopkins, Stevenson, and Weil have reported varying results. Hopkins states that lower figures are often found in cases of syphilis than in any other disease, with the exception of non-syphilitic meningitis.

The relation of whole blood sugar to cerebrospinal sugar has been studied by a few workers. Bang states that they are present in the same percentage. Hopkins, using Bang's method, found the blood sugar to be 10 mg. higher. Polonowski and Duhot found the ratio of cerebrospinal fluid to blood sugar to be equal to 1:1. Servantie states that the ratio varies from 0.4:1 to 1:1. Myers and Fine studied the spinal fluid in 15 cases in which there were varying degrees of nitrogen retention, and found the sugar of the cerebrospinal fluid to be 57 per cent of that in the blood. In cases without diabetes Rusznyák and Csáki found the sugar in the spinal fluid to be much less than that of the blood plasma, the average difference being 54 mg. This might be construed as contradictory to Mestrezat's theory, which has already been mentioned. Many investigators did not consider the blood sugar at all, and made no attempt to control the alimentation of their patients. Polonowski and Duhot found higher values when the spinal fluid was withdrawn from three to five hours after the last meal than when it was withdrawn ten hours after the last meal. Increased values have been found in the cerebrospinal fluid in cases of diabetes. Thalhimer and Updegraff could find no increase, however, until the blood sugar reached 190 mg. and believed this might signify a threshold level for passage of sugar into the spinal fluid. They estimated the value of the sugar in the spinal fluid at 45 per cent of that found in the blood. Normal blood sugar is generally given as ranging from 90 to 120 mg. for each 100 c.c. of blood.

TECHNIC FOR ESTIMATION OF SUGAR CONTENT

In this study the concentration of sugar, both in the blood and cerebrospinal fluid, was determined by the Rothberg and Evans' modification of the Folin and Wu method. This method was chosen with a view to accurate determination, and the work was personally performed. The blood protein was precipitated as recommended by Folin and Wu, but for the cerebrospinal fluid the procedure was altered. To 2.0 c.c. of fluid were added 6.0 c.c. of distilled water, 1.0 c.c. of 10 per cent sodium tungstate, and 1.0 c.c. of two-thirds normal sulphuric acid. This is considered sufficient to precipitate all the protein and permits the use of the customary standard No. 1 of the Folin and Wu method for both blood and spinal fluid, thereby eliminating the preparation of a standard of one-half this strength, as suggested by Foster. The unknown is diluted until the intensity of color matches that of the known. This is generally obtained at 30 c.c.

The blood and spinal fluid of the normal subjects were collected simultaneously about sixteen hours following the last meal. This was also true of most of the patients who had syphilis without involvement of the central nervous system. When the central nervous system was affected the spinal

TABLE I

INITIAL CONCENTRATION OF SUGAR IN THE SPINAL FLUID AND BLOOD IN 175 CASES

TYPE OF CASE	SPINAL FLUID			BLOOD			REMARKS
	CASES	RANGE OF SUGAR CONCENTRATION, MG.	AVERAGE SUGAR CONCENTRATION, MG.	CASES	RANGE OF SUGAR CONCENTRATION, MG.	AVERAGE SUGAR CONCENTRATION, MG.	
Controls (non-syphilitic)	23 1	56 to 77	67 45	19 1	84 to 97	90 66	Hypoglycemia (?)
Latent syphilis	19 1	53 to 69	61 100	10 1	80 to 103	90 276	Pancreatitis
Primary and secondary syphilis	4	59 to 70	64	4	81 to 90	86	
Congenital syphilis	1		56	1		99	
Vascular neurosyphilis	2	52 to 59	56	2	70 to 136	103	
Serologically negative neurosyphilis	1		63	1		97	
Undifferentiated neurosyphilis	37 1	45 to 71	58 82	32 1	60 to 129	95 109	Glycosuria several times
Asymptomatic neurosyphilis	12	42 to 68	56	11	67 to 122	100	
Asymptomatic meningeal neurosyphilis	12	47 to 74	62	8	85 to 126	94	
Tabes dorsalis	59	41 to 73	57	50	64 to 156	93	
Juvenile paresis	2	46 to 55	50	2	78 to 84	81	
General paresis	9	41 to 67	57	7	78 to 105	94	

fluid was removed at the time intraspinal treatment was given (Table I). This followed the administration of arsphenamine on the previous day and the ingestion of a light breakfast, as a rule, on the morning of intraspinal treatment. The length of time without food made some difference in the content of sugar in the blood, but no alteration in the content of sugar in the spinal fluid could be ascertained.

Precipitation was carried out very soon after the spinal fluid was withdrawn. If the sugar could not be determined immediately, a few drops of toluol were added to the filtrate, and it was placed in a refrigerator. This precaution was taken to avoid possible glycolysis, as Csáki reported 50 per cent loss of reducing substance in three hours at room temperature in the case of cerebrospinal fluid from a patient with diabetes. Löwy found no decrease in from twenty-four to forty-eight hours at room temperature and at 37° C. Stevenson obtained the same result after the fluid had stood for two or more days. The last two authors were not considering the spinal fluid in cases of diabetes.

The supposedly normal patient with low concentration of sugar in the blood and spinal fluid had no demonstrable abnormality except fibromyoma of the uterus. This was the only case in the entire series with subnormal findings in both blood and spinal fluid. This condition has been experimentally produced by Polonowski and Duhot, as will be mentioned later. At operation

and necropsy pancreatitis was discovered in the case of latent syphilis with high concentration of sugar in the blood and spinal fluid. It is interesting to note that urinalyses, taken up to five days before sugar determinations were made, showed no sugar. In the case of neurosyphilis with high concentration of sugar in the spinal fluid and normal concentration in the blood there was a slight trace of sugar in the urine on several occasions. This patient was Jewish, and there may have been some disturbance in carbohydrate metabolism, although the glycosuria was thought to be of renal origin. In the cases without syphilis, used as controls, there was no demonstrable organic lesion of the nervous system.

RELATION OF THE CONCENTRATION OF SUGAR IN THE SPINAL FLUID TO SEROLOGIC FINDINGS

Correlation of the concentration of sugar in the spinal fluid in cases of neurosyphilis with serologic findings has been attempted by a few workers. Borberg found especially low values in cases with pleocytosis. Kahler states that the cell count makes no difference, although he had no cell count above 55 lymphocytes for each cubic millimeter. Moates and Keegan found no connection between the sugar content and the other findings. Wittgenstein states that many factors must be considered in the interpretation of results and presents noteworthy tables. In general he assumes that in cases of tabes dor-

TABLE II

CONCENTRATION OF SUGAR IN THE SPINAL FLUID IN CASES OF NEUROSYPHILIS

TYPE OF CASE	HIGH CELL COUNT*, MG.	NORMAL CELL COUNT, MG.	COLLOIDAL BENZOIN REACTION IN FIRST ZONE, MG.	COLLOIDAL BENZOIN REACTION IN OTHER ZONES, MG.
Vascular neurosyphilis		56	56	
Undifferentiated neurosyphilis	56	59	54	60
Meningeal neurosyphilis	64	64	64	64
Asymptomatic neurosyphilis	60	59	52	59
Tabes dorsalis	53	58	54	57
Juvenile paresis		53	53	
General paresis (1 case)	49	60	62	51

*More than 10 cells for each cubic millimeter was considered abnormal.

salis and undifferentiated neurosyphilis, a high cell count with low concentration of sugar in the spinal fluid denotes a meningitic process; that a high cell count in cases of tabes dorsalis with high concentration of sugar denotes a complicated cerebral process, and that a high cell count in cases of neurosyphilis with high concentration of sugar may be due to allergy. He supports this assumption by stating that in one such case monoplegia developed during treatment with arsphenamine. He states that the sugar content seems to have diagnostic significance in cases of cerebral inflammation, in which he finds higher percentages. His tables must be studied to be appreciated. In Table II are shown the results of my cases with respect to cell count and col-

loidal benzoin reaction. The percentages represent the average concentration of sugar in the spinal fluid for the various groups.

There is a slight tendency to lower values along with high cell count, and also with colloidal benzoin reactions in the first zone.

INFLUENCE OF ALIMENTARY HYPERGLYCEMIA

Since it was found by Hess and Pötzl that the oral ingestion of iodides was followed by their appearance in the cerebrospinal fluid, only in cases of meningitis, since increased iodide values in cases of syphilitic meningitis were found by Osborne, and since increased nitrate values, after administration of the salt, were found by Mestrezat and Gaujoux, studies were made of the effect of alimentary hyperglycemia on the sugar in the spinal fluid in cases of neurosyphilis. Kelley increased the blood sugar of rabbits by intravenous injection of glucose and found no increase in the sugar in the spinal fluid. Polonowski and Duhot produced hyperglycemia by subcutaneous injection of epinephrin and produced an increase in the sugar in the spinal fluid. They considered, however, that the epinephrin may have introduced an added factor by possibly altering the permeability of the choroid plexus. They produced alimentary hypoglycemia by administering large amounts of glucose and saccharose, and found a decrease in the sugar in the fluid. I first determined the sugar in the blood and spinal fluid to determine the patient's normal. Two weeks later I administered orally 100 gm. of glucose fifteen minutes, thirty minutes, or one hour before the withdrawal of blood and spinal fluid, using the various intervals on different occasions. The blood and spinal fluid were withdrawn almost synchronously. Glucose was given to 21 patients for a total of 30 administrations. The content of sugar in the blood was highest after the half-hour interval, reaching a peak of 186 mg., but without a definite increase in the sugar in the spinal fluid at any time. In 2 cases there was no change; in 15 cases there was an average increase of 6 mg., and in 13 cases an average decrease of 5 mg. The greatest increase, 10 mg., was in a case of meningitis. The general averages of various types of neurosyphilis were approximately identical. The highest concentration of sugar in the blood, 186 mg., was associated with an increase of but 5 mg. The negative result may be due to the fact that the threshold value of 190 mg., as found by Thalhimer and Updegraff, was not reached.

INFLUENCE OF TREATMENT

A majority of the patients with neurosyphilis had had a certain amount of treatment, but there was no appreciable difference in the findings in these cases and those untreated. The patients were being treated by intravenous and intraspinal injections of arsphenamine by the Swift-Ellis-Ogilvie method, with iodides, and mercury or bismuth. To determine the behavior of the sugar in the spinal fluid under treatment, determinations were made on 2 occasions in 24 cases, and on 3 occasions in 2 cases. This is exclusive of patients who received glucose. The determinations were made at intervals of from two weeks to five months. In 1 case the result was the same at 2 examinations; in 15 cases there was an average increase of 5 mg., and in 8 cases

there was an average decrease of 7 mg. In the 2 cases with 3 examinations (at intervals of two weeks) the variation in results did not exceed 3 mg. The length of time between examinations seemed to make no difference. Kelley found an average concentration of sugar in the spinal fluid of 21 mg. in cases of neurosyphilis without treatment and an average of 62 mg. in cases in which treatment was given. Biach, Kerl and Kahler found from 40 to 90 mg. in cases without treatment and as high as 340 mg. in a case following treatment. These authors did not state specifically that the figures before and after treatment were obtained in the same cases. Wittgenstein obtained marked increase in sugar in the spinal fluid in cases of neurosyphilis after intravenous injection of neoarsphenamine, with and without spinal drainage. Alpers, Campbell, and Prentiss found the content decreased in cases of paresis following treatment, and Weston found slightly higher values.

PROGNOSTIC VALUE OF THE DETERMINATION OF SUGAR IN THE SPINAL FLUID

This phase of the subject has received scant attention. Wittgenstein believes increased values for sugar in the spinal fluid signify cerebral involvement, which might increase the gravity of the situation. If the colloidal benzoin reaction in the first zone signifies cerebral parenchymatous involvement, as Osborne believes, my findings are at variance with those of Wittgenstein. My patients have been observed as long as one and one-half years. Those who on the basis of clinical and serologic findings promise to be resistant to treatment tend to have a decreased concentration of sugar in the spinal fluids with a greater range in values. There is, however, no apparent difference between those with low and those with high values. Further observation will be necessary to determine the prognostic value of these levels.

DISCUSSION

There are several sources of error in making determinations, some of which have been mentioned by Polonowski and Duhot: the blood which is examined is from the peripheral vessels and may differ from that in the choroid plexus; the determinations have been made on whole blood and not on plasma; and the filtrate may contain reducing substances other than sugar. In fact, Mestrezat goes so far as to condemn all methods of precipitating blood protein except by means of mercuric acid sulphate. Due to the small variation, the method of determining sugar must be as accurate and sensitive as possible. Division of cases of neurosyphilis into distinct groups is inaccurate on account of the manifold involvement. Comparison of the sugar content of the spinal fluid with cytologic and serologic findings is inaccurate, since a high cell count may fall markedly and a colloid benzoin reaction in the first zone may disappear under intraspinal medication with little or no change in the sugar content.

With regard to the etiology of decrease in the sugar content of the spinal fluid, it is possible that, as suggested by Wilcox and Lyttle, disease of the meninges or choroid plexus may prevent the glucose from entering the spinal fluid. The varying results could be explained by difference in the degree of involvement of the portion of the meninges that covers the choroid plexus. It would be interesting to study the choroid plexus at necropsy in

cases of neurosyphilis in relation to degree of involvement and also in regard to glycogen content, to which attention has been directed by Yoshimura. Kelley, on the basis of inoculation experiments in vitro, believes that the spirochete utilizes the sugar of the spinal fluid as food. The difficulty encountered in demonstrating the organisms in the spinal fluid would more or less preclude their presence in sufficient numbers to make any appreciable change in the concentration of sugar.

CHLORIDES OF THE CEREBROSPINAL FLUID

There are fewer reports of examination of the chlorides of the cerebrospinal fluid than of the determination of sugar, and less variation in the estimation of normal values. The results range from a minimum of 610 mg. to a maximum of 750 mg. for each 100 c.c. of spinal fluid. The normal chlorides of whole blood range from 550 to 600 mg. for each 100 c.c. These figures are

TABLE III

CONTENT OF CHLORIDES IN SPINAL FLUID AND BLOOD

TYPE OF CASE	CASES	SPINAL FLUID		BLOOD	
		RANGE OF CHLORIDES, MG.	AVERAGE AMOUNT OF CHLORIDES, MG.	RANGE OF CHLORIDES, MG.	AVERAGE AMOUNT OF CHLORIDES, MG.
Normal	11	710 to 775	731	440 to 460	486
Latent syphilis	8	710 to 740	727	460 to 620	510
Primary and secondary syphilis	5	710 to 738	723	420 to 520	455
Congenital syphilis	1		730		480
Undifferentiated neurosyphilis	37	660 to 780	739	430 to 670	513
Asymptomatic neurosyphilis	21	660 to 760	730	426 to 620	522
Tabes dorsalis	41	685 to 775	734	410 to 620	510
General paresis	4	730 to 175	739	440 to 520	485

expressed in terms of sodium chloride. Flockenhaus and Fonseca found them as high as 900 mg. in cases of paralysis. Csáki found them increased in cases of syphilis and "metasyphilis." He observed decreased values in one case of syphilitic meningitis, which increased following treatment. Depisch and Richter-Quittner found increased values in cases of syphilis.

TECHNIC FOR THE ESTIMATION OF CHLORIDE CONTENT

Estimation of chlorides was made on the protein-free filtrates such as were used in determining the content of sugar. The following is the method employed:* The silver nitrate reagent is prepared by dissolving exactly 2.905 gm. of silver nitrate in about 100 c.c. of water and dissolving 30 gm. of ferric ammonium sulphate in about 300 c.c. of water, and by adding these solutions together, with 500 cc. of concentrated nitric acid, in a 1,000 c.c. volumetric flask, and by diluting to the mark with water; 1 c.c. equals 1 mg. of sodium chloride.

*The method is that of Whitehorn, as modified by W. G. Karr, Chief Chemist, Philadelphia General Hospital.

The ammonium thiocyanate reagent is prepared by dissolving about 2.7 gm. of ammonium thiocyanate in about 1 liter of water. This is placed in a 5 c.c. microburette. In a 50 c.c. porcelain casserole are placed 10 c.c. of water and 10 c.c. of the silver nitrate reagent. This is then titrated with the thiocyanate from the burette. The end-point is the first reddish-brown color that persists for fifteen seconds. This titration is repeated for confirmation. Two hundred times the amount of thiocyanate solution required for the titration is diluted to 1 liter; 5 c.c. of this solution is then equal to 10 c.c. of the silver reagent and is equivalent to 10 mg. of sodium chloride.

Ten cubic centimeters of the protein-free filtrate is transferred to a 50 c.c. porcelain casserole and 10 c.c. of the silver nitrate reagent added. It is allowed to stand five minutes and then is titrated with the ammonium thiocyanate solution from the microburette until a slight reddish-brown persists for at least fifteen seconds.

If r = quantity of thiocyanate used (in cubic centimeters)
and x = quantity of sodium chloride (in milligrams for each 100 c.c. of blood)
then x = 200 (5–r)

In the case of the cerebrospinal fluid the filtrate must be diluted by an equal amount of water or the results divided by two, since the filtrate is twice as concentrated as that from the blood.

This method was chosen because it permitted determinations to be made on the same filtrate as was used for the estimation of sugar. One hundred thirty-one determinations were made on the blood and spinal fluid of 100 patients. There was a little variation from time to time, the following being a typical example of a case of meningeal neurosyphilis:

Date	Whole Blood Chlorides, mg.	Spinal Fluid Chlorides, mg.
June 10, 1924	560	740
June 24, 1924	580	755
July 8, 1924	520	730

In general, the range of variation is greater in the blood than in the cerebrospinal fluid, as is evident in Table III which includes cases both with and without treatment. Specimens were examined at different times during treatment, and all results were within normal limits.

SUMMARY

There is a definite but small decrease, with rather wide range of values, in the reducing substance of the cerebrospinal fluid in cases of neurosyphilis, as determined by Rothberg and Evans' modification of the Folin and Wu method. Simultaneous determination of blood sugar is of assistance in a small percentage of cases. There is a slight normal variation in the content of sugar in the spinal fluid but not as great as in blood sugar. Length of time without food made some difference in the level of sugar in the blood, but apparently none in that of the cerebrospinal fluid.

There is a slight tendency toward low values for sugar in the spinal fluid along with high cell count and in cases with colloidal benzoin reaction in the first zone. The regular comparative tabulations of Wittgenstein could not be

duplicated. Alimentary hyperglycemia up to 185 mg. produced no increase of sugar in the spinal fluid. There was no alteration in sugar content under treatment in cases followed as long as five months. The extremely low values for sugar in the spinal fluid in cases of neurosyphilis and the marked rise following treatment, as noted by some authors, could not be confirmed. No information was obtained that would definitely aid in prognosis, although observation has lasted one and one-half years. Careful observation over years with repeated determinations of the content of sugar in the spinal fluid may prove valuable.

There is a variation in the chloride content of the cerebrospinal fluid which is not as great as in the chlorides of the whole blood. In all the cases of neurosyphilis, the spinal fluid showed a normal chloride content.

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THE USE OF ISOPROPYL ALCOHOL IN THE PREPARATION OF WASSERMANN ANTIGENS*

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COMPARATIVELY recently isopropyl alcohol has become available in quantity at reasonable prices. Because of the high tax on ethyl alcohol and the onerous restrictions connected with its withdrawal and use, it is in many instances being replaced by isopropyl alcohol, which is less costly and is free from all regulation, since it is nonpotable.

These considerations have led the author to undertake an investigation of the possibility of preparing Wassermann antigens by use of isopropyl alcohol instead of ethyl alcohol. The close similarity in physical properties of the two alcohols indicated the probable success of the investigation, while the results obtained show isopropyl alcohol to be actually superior to ethyl alcohol for this purpose. It proved to be a better solvent for the antigenic substances of normal tissue than ethyl alcohol.

In this work two types of antigen were prepared: the simple alcoholic extract of heart muscle and the alcohol ether soluble and acetone insoluble antigen as used by the Hygienic Laboratory. Results were controlled by

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making identical preparations from the same hearts, using 95 per cent ethyl alcohol for the extraction.

The simple alcoholic extract prepared by use of isopropyl alcohol was much higher in antigenic power than that prepared by use of ethyl alcohol. While it required twenty-five antigenic units to give partial inhibition of hemolysis with normal sera, in the case of the antigenic extract prepared with isopropyl alcohol, only ten units were required in the case of the ethyl alcoholic extract.

In the preparation of the acetone insoluble antigens, isopropyl alcohol was found to give approximately a 10 per cent greater extraction of acetone insoluble material than ethyl alcohol. The antigenic power of the methyl alcohol and ether solutions of the acetone precipitates was equal in the two cases. Even 0.20 c.c. of the antigen prepared with isopropyl alcohol failed to give any inhibition of hemolysis with normal sera, while some inhibition was apparent with 0.16 c.c. of the antigen prepared with ethyl alcohol.

EXPERIMENTAL

Simple Alcoholic Extracts.—A fresh beef heart was freed from fat and connective tissue and the heart muscle ground in a meat grinder. The ground muscle was thoroughly mixed, and two 200 gm. portions were weighed out. These were transferred to 1200 c.c. flasks and extracted for two weeks at 37° C., the one with one liter of 91.1 per cent isopropyl alcohol, the other with one liter of 95 per cent ethyl alcohol. During extraction the flasks were well shaken, three times daily.

After extracting for two weeks, the solutions were filtered and the filtrates preserved in tightly-stoppered bottles in the ice box. On cooling, both solutions deposited some material, the isopropyl alcohol solution depositing considerably more than the ethyl alcohol solution. The isopropyl alcohol solution retained a considerably higher color than the ethyl alcohol solution.

These simple alcoholic extracts were tested for antigenic and anticomplementary properties in the usual manner, in an antisheep hemolytic system, by using two units of complement, two units of amboceptor, and pooled strongly positive and pooled normal sera, respectively, with graded amounts of antigen. Incubation for fixation of complement was at 37° C., for thirty minutes. After adding amboceptor and sheep cells, incubation at 37° C. was continued for thirty minutes; and the tubes were then allowed to stand overnight at 15° C. before reading. Results of these tests appear in Table I.

A comparison of the data present in Table I shows that even 0.004 c.c. of the isopropyl alcohol antigen gives total inhibition of hemolysis with positive sera, while no inhibition with normal sera appears until amounts in excess of 0.10 c.c. of the antigen are used. We thus have a ratio of 0.10/0.004 equals 25/1.

In the case of the ethyl alcohol antigen, it requires 0.01 c.c. to give total inhibition with positive sera, and amounts above 0.10 c.c. show inhibition with normal sera. The ratio here is thus only 0.10/0.01 equals 10/1.

Acetone Insoluble Antigen.—The preparation and preliminary extraction of the heart muscle, in the case of the acetone insoluble antigens, was the

TABLE I

TITRATION OF SIMPLE ALCOHOLIC ANTIGENS

ANTIGEN TAKEN C.C.	POSITIVE SERUM HEMOLYSIS		NORMAL SERUM INHIBITION	
	ISOPROPYL ANTIGEN	ETHYL ANTIGEN	ISOPROPYL ANTIGEN	ETHYL ANTIGEN
0.000	Total	Total	None	None
0.004	None	75%	"	"
0.006	"	50%	"	"
0.010	"	None	"	"
0.020	"	"	"	"
0.040	"	"	"	"
0.060	"	"	"	"
0.080	"	"	"	"
0.100	"	"	"	"
0.140	"	"	60%	10%
0.160	"	"	Total	30%
0.200	"	"	"	Total

same as for the simple alcoholic extracts except that one liter of alcohol was used for each 100 gm. of ground heart muscle.

After extracting at 37° C. for two weeks and filtering, the alcoholic extracts were evaporated at room temperature before a fan. The residues were then taken up with ether and the solutions transferred to stoppered bottles and left overnight for sedimentation.

The clear ethereal solutions were decanted, evaporated to a volume of 20 c.c., and treated with 200 c.c. of acetone, each, for precipitation of the lipoids. The vessels were covered and allowed to stand overnight, in the ice box, to collect the precipitates. The acetone was decanted, and the precipitates were allowed to dry to the usual sticky consistancy.

The yield of acetone insoluble lipoids obtained from 100 gm. of heart muscle, extracted with 1000 c.c. of 91.1 per cent isopropyl alcohol, was 3.5 gm. From 100 gm. of heart muscle 3.2 gm. of lipoids, extracted with 1000 c.c. of 95 per cent ethyl alcohol were obtained.

Stock antigen solutions were prepared from these in the usual manner by dissolving 0.30 gm. of lipoids in 10 c.c. of methyl alcohol and 1 c.c. of ether.

Titrations were carried out in the same manner as for the simple alcoholic extracts with results as shown in Table II.

TABLE II

TITRATION OF ACETONE INSOLUBLE ANTIGENS

ANTIGEN TAKEN C.C.	POSITIVE SERUM HEMOLYSIS		NORMAL SERUM INHIBITION	
	ISOPROPYL ANTIGEN	ETHYL ANTIGEN	ISOPROPYL ANTIGEN	ETHYL ANTIGEN
0.000	Total	Total	None	None
0.004	None	None	"	"
0.006	"	"	"	"
0.010	"	"	"	"
0.020	"	"	"	"
0.040	"	"	"	"
0.060	"	"	"	"
0.080	"	"	"	"
0.100	"	"	"	"
0.140	"	"	"	"
0.160	"	"	"	Trace
0.200	"	"	"	10%

An examination of the results shown in Table II shows the antigenic powers of the two preparations to be identical. The antigen prepared with ethyl alcohol shows some inhibition of hemolysis when normal sera are used in amounts above 0.14 c.c., while the isopropyl alcohol product shows none in any of the amounts tested.

CONCLUSIONS

1. Isopropyl alcohol is superior to ethyl alcohol for the preparation of Wassermann antigens of the simple alcoholic type. It yields a product of superior antigenic power and shows relatively less anticomplementary action.

2. Isopropyl alcohol is superior to ethyl alcohol for the production of acetone insoluble antigens because it gives a more complete extraction of the acetone insoluble lipoids of normal tissues. The antigen produced is equal in antigenic power to that produced by use of ethyl alcohol and is somewhat superior as regards anticomplementary behavior with normal sera.

3. The use of isopropyl alcohol for this purpose is also to be recommended over the use of tax-paid ethyl alcohol because it is less costly and is not subject to troublesome regulations.

SPIROCHETAL BRONCHITIS
REPORT OF A CASE SUCCESSFULLY TREATED WITH ARSPHENAMINE*

By John W. Visher, M.A., M.D., Twin Falls, Idaho

THE existence of pulmonary infections due to the spirochete of Vincent and to the fusiform bacillus has only recently been recognized. Credit is due to Pilot and Davis[1] for their pioneer work in this field, and those interested in this subject are referred to their exhaustive article for a complete discussion and bibliography. Within the last year several other papers have appeared on this subject, the most important of which is one by Kline and Berger.[2] Most of the cases reported have exhibited pulmonary abscesses and pulmonary gangrene. The following case is, therefore, of interest because of its gradual onset and because abscess formation did not occur.

REPORT OF CASE

Mrs. N. F. S., aged thirty-eight, developed acute bilateral pyosalpinx following the birth of her eighth child on September 21, 1924. A laparotomy was performed October 23, 1924, and both tubes and the left ovary were removed and drainage instituted. I saw her in consultation before the operation and learned that her previous health had been quite good. A careful physical examination at that time revealed no pulmonary pathology. There

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From the U. S. Veterans' Hospital, Waukesha, Wisconsin.
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was moderately severe pyorrhea alveolaris. Ether was given and no immediate complications followed, but a bronchitis gradually developed and became progressively worse until January 20, 1925, when I saw her again in consultation. She had lost fifty pounds in weight, had a severe productive cough, and looked decidedly ill. There had been an irregular temperature, often higher in the morning, reaching 102° at times. Her pulse was quite rapid, varying between 100 and 110. The white count was 12,500; the red count was 3,500,000; and the hemoglobin was 65 per cent. Her breath and sputum were very offensive in odor. The latter was mucopurulent, very light brown in color, and contained small flecks of blood. Examination of the chest revealed many moist, moderately coarse râles scattered diffusely throughout both lungs, not more numerous in the apices. Breath sounds were roughened, but there were no evidences of large areas of consolidation. Radiograms showed mottling throughout both lungs, resembling that seen in influenzal pneumonia, but no abscesses. Repeated sputum examinations for tubercle bacilli were negative, but large numbers of fusiform bacilli and of Vincent's spirochetes, together with streptococci and pneumococci, were found. Three intravenous injections of neoarsphenamine (0.15, 0.3, and 0.6 grams) were given in the course of two weeks with the result that the cough abated, the râles disappeared, the sputum became scanty and normal in character, and no more fusiform bacilli or spirochetes could be found. She looked and felt much better, and her appetite improved greatly. Two weeks later she developed a slight cough secondary to a rhinitis. A few spirilla were found in the sputum and an intramuscular injection of sulpharsphenamine was given to guard against a recurrence of the pulmonary infection. There has been no return of the bronchitis to date (December 1, 1925), and she writes that she never felt better. Her present weight is 128 lbs., a gain of 28 lbs. in ten months.

COMMENT

The similarity of this case to one of rapidly progressing pulmonary tuberculosis is apparent. In fact, this syndrome has actually been called pseudotuberculosis. Certain important differences, however, were noted. The temperature curve was not typical, being higher in the morning, and there were no night sweats. The classical findings of consolidation in the apices were absent; and the râles, which were somewhat coarser than those heard in tuberculosis, were scattered diffusely over the whole chest. The radiograph also was not typical as the apices were too clear, and the mottling was too general. In spite of these atypical findings, the diagnosis was in doubt until fusiform bacilli and spirochetes were noted while examining a smear for tubercle bacilli.

It was found that the spirochetes did not stain well with methylene blue, but the fusiform bacilli stained readily with this dye. The examiner should keep on the watch for the latter organism while searching for tubercle bacilli. The spirochetes can be readily demonstrated if a thin smear is stained deeply with carbolfuchsin and examined before decolorization. They were very numerous in this case before treatment was instituted. As a few organisms

are often present in saliva, the sputum should either be washed or care exercised to make the smear from the center of a mass of purulent sputum.

Pyorrhea alveolaris has been mentioned by several authors as the source of the pulmonary infection and was present in this case. Proper oral hygiene is, obviously, an important prophylactic measure. Local treatment to the patient's gums was instituted promptly.

The rapid improvement in the bronchitis following neoarsphenamine administration was gratifying. Since pulmonary abscess and gangrene respond much less readily, it is important that an early diagnosis be made and that treatment be instituted promptly.

SUMMARY

A case of purulent bronchitis due to Vincent's spirochete and to the fusiform bacillus is reported in which prompt recovery followed the administration of neoarsphenamine intravenously. The importance and the simplicity of the early recognition of this infection are emphasized.

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REPORT OF A CASE OF COCCIDIOIDAL GRANULOMA WITH AUTOPSY FINDINGS*

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SINCE Dickson's[1] review of the literature on coccidioidal granuloma, twelve new cases[2] have been reported. The majority of the new cases (seven) were observed in California; the others were one from Missouri, one from Charleston, South Carolina, and one from Kansas. The latest case reported was from Chicago. This case came, however, originally from California.

We wish to report a new case which we observed in San Jose, which ended fatally and was interesting in so far as we were able to obtain a pure culture of the fungus from the blood stream during life. This is the first case to our knowledge in which the organism could be demonstrated in the blood.

HISTORY

Mr. T. H., aged sixty-one, was a cement and plaster contractor. His father died of apoplexy; one sister died of cancer of the stomach; otherwise his family history was negative. When a small child he had an almost fatal infection following the opening of a boil and was many months in recovering. He had

*From the Laboratories of San Jose Hospital and Agnew State Hospital.
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two tapeworms removed (type undetermined). Two years before he had an acute attack of cholecystitis, which lasted for about one month, but he was not confined to bed.

His present illness began the first week in September, 1923, while on a trip to the southern part of California for a rest. He developed a cold with a slight cough and some fever, but in spite of this he did not go to bed until ordered about three weeks later. He was bedridden for six weeks, during which time he ran a temperature ranging from 99 to 101° with marked general malaise and debility but with no special complaint except insomnia and a slight cough.

Physical Examination.—A physical examination made on October 11, 1923, showed the following: the eyes reacted to light and accommodation, conjunctiva clear, tongue slightly coated, tonsils and pharynx inflamed, cervical glands and thyroid not enlarged. The lungs were negative with the exception of a few subcrepitant râles scattered over the chest both front and back. Voice and breath sounds were normal. The heart was not enlarged; there were no murmurs, neither irregularity nor arrhythmia. Abdomen was soft, liver not palpable; spleen was palpable and of a doughy consistency. The prostate gland was not enlarged; inguinal glands were not palpable. The central and peripheral nervous systems normal. The Wassermann and Widal tests and the urinary findings were negative. Blood examination showed 7,600 leucocytes. No tubercle bacilli were found in the sputum in spite of numerous examinations.

Fig. 1.—Pure culture Coccidiodes imitis. Hanging drop preparation form bouillon culture. Oil 1/12 Oc. 4.

Clinical Course.—During October the patient developed a pain in the left hip, the right ankle, and the thumb accompanied by a moderate degree of swelling in the thumb and ankle but without redness. The pain was al-

most continuous, increased by motion, and was relieved by salicylates. Early in December there developed on the nose and the lower right palpebra near the inner canthus, a small nodular papule, almost cartilaginous to touch and about the size of a bean. This broke down in the center and presented a crater-like ulcer which discharged pus. A month later, several similar nodules appeared on the extensor surface of the arms. These did not break down but remained hard, red, nodular papules about 1 cm. in diameter.

The swellings were painful and on opening proved to be subperiosteal abscesses. In spite of curettage and drainage they did not heal. About the same time there appeared on his head, 4 or 5 fluctuating nodules, which also proved to be subperiosteal abscesses, painful only on pressure. These likewise failed to heal when treated surgically. From the pus of all the above lesions a pure culture of a fungus-like organism, resembling Coccidioides imitis, was obtained. The pus contained the typical parasites but no fungi. A description of the microorganism follows later.

In the latter part of January, headache and some rigidity of the neck developed, this persisting throughout the course of the disease. With exception of the anorexia from the acute illness, symptoms of the gastrointestinal tract were lacking. The respiratory system, with exception of the slight, hacking cough which subsided and did not reappear, failed to show any symptoms throughout the latter course of the disease. Blood examination on January 1, showed 75 per cent hemoglobin, 9,500 leucocytes, 64 per cent neutrophiles, 4 per cent eosinophiles and 32 per cent lymphocytes. Another one on January 2, showed 7,300 leucocytes, 70 per cent neutrophiles, 9 per cent eosinophiles, and 21 per cent lymphocytes. A blood culture taken at this time was negative. Urine examinations showed a trace of albumin and a few hyaline casts. The blood pressure was low throughout. Finally tachycardia and a toxic myocarditis developed. The central nervous system showed no involvement until late in the disease (about three weeks before death) when the patient developed a mental sluggishness merging into mild delirium and coma. Four days before death a blood culture was taken which, after six weeks of incubation, developed a growth of the same fungus isolated from the abscesses.

The treatment was purely supportive and symptomatic. In the beginning sedative expectorants and salicylates were given by mouth and intravenously; the lesions were treated with iodine, mercurochrome, gentian violet, and potassium permanganate but without response. A vaccine was made from the microorganism isolated in pure culture from the lesions and given in increasing doses up to 10 c.c. but without results. Practically no reaction either general or local was observed during the vaccine treatment.

Radiation with ultraviolet rays gave negative results when applied locally and generally. The patient failed rapidly and died February 27, 1924.

AUTOPSY FINDINGS

A complete autopsy was performed one hour after death, showing the following points of interest:

Greatly emaciated male body. On the right side of the nose and the lower eyelid there was a dry, pale grayish-red scab of about 2 × 1 cm. Upon

removal of the scab a rather sharply outlined ulcer was disclosed containing a thick gray-yellow mucoid pus. Its borders were not infiltrated and showed no signs of inflammatory reaction. On removing the pus a pale grayish-red, almost smooth ulcer remained, apparently involving only the corium.

On the frontal, left parietal, left and right temporal regions the integument was covered with dry-brownish-red scabs. Removal of the scabs brought to view partially healed operative incisions about 2 cm. long. The borders of the incisions were slightly infiltrated, and on pressure a gray mucoid pus could be evacuated from all of them. The largest quantity of purulent material was obtained from the incision over the right temporal region. On palpating the underlying bony structures, distinct depressions could be noted.

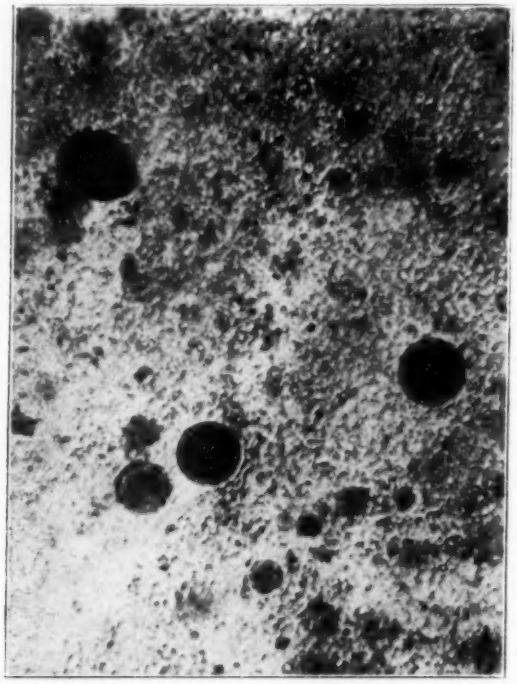

Fig. 2.—Typical spore-like microorganisms from pus of thumb. Erythrosin glycerin sodium acetate. Oil emersion Oc. 4.

The skull cap showed the following lesions: Left upper third of coronal suture between marginal portion of frontal and parietal bones disclosed a subperiosteal and osteomyelitic abscess 20 × 10 × 6 mm., forming an irregularly outlined ragged cavity extending in part beneath the external tabula into the frontal and parietal bones. The anterior part of left parietal bone, 2 cm. above the squamous suture showed an abscess about 3 mm. in diameter, cone shaped, extending into the diploë, not perforating through the internal tabula. The posterior part of the left parietal os 2 cm. below the sagittal suture showed an abscess 10 × 7 × 2 mm. The lower posterior part of parietal os showed a horse-shoe-shaped abscess 22 × 10 × 3 mm. perforating through the external tabula. The dura beneath was covered with granulation tissue. Two centimeters above the left squamous suture was a superficial ero-

sion of the external tabula 10 × 5 mm. The right upper portion of occipital suture near the lambda suture showed two subperiosteal abscesses, each with superficial erosion of external tabula 5 × 5 × 1 mm. and 5 × 2 mm. The left occipital suture disclosed an abscess 22 × 12 × 3 mm. with multiple punctiform perforations into the internal tabula. The lower posterior portion of left parietal bone showed, just above the incisura parietalis, a nonperforating abscess 12 × 9 mm.

Over the right medial malleolus there was an ulcer about 4 × 2 cm., its borders sharply outlined, but without any infiltration. On pressure about 20 c.c. of slightly bloody, gray-white pus was evacuated from the underlying structures. Enlargement of the abscess cavity showed that the subcutaneous

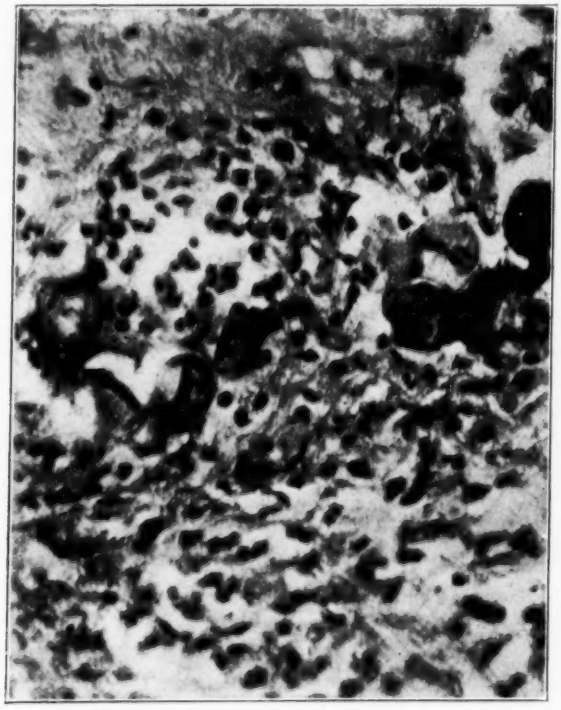

Fig. 3.—Section from lung showing several spore-like forms. Leitz Obj. 4 and Oc. 4.

tissue had been transformed into a grayish-yellow mucoid pus. The ligaments were surrounded with pus but not loosened. The periosteum of the medial malleolus was necrotic; the spongiosa was soft, friable, and infiltrated with pus; the articulatio-talocruralis was not involved and the cartilage was smooth and glistening.

Over the dorsum of the right foot there was a fluctuating area about the size of a dollar with the integument slightly raised and of a light smoky-gray. In the center appeared a small opening which on pressure yielded a small quantity of gray-yellow mucoid pus. Incision revealed a subcutaneous abscess. The underlying tendons of the long extensor muscles of the leg were bathed in pus. The second, third, and fourth spatia interossea were filled

with pus. The short extensor muscles of the toes were necrotic and transformed into a smeary-gray-yellow material. The periosteum of the second, third, and fourth metacarpal bones was lacking; the compacta soft and the spongiosa friable.

Over the metacarpophalangeal joint of the right thumb there was a single incision about 2 cm. long from which gray-yellow pus was obtained on pressure. Enlargement of the opening showed the capsule of the joint to be covered with pus. The surrounding muscles were pale, grayish-red and infiltrated with pus. The periosteum of the distal end of the first metacarpal and of the proximal end of the first phalanx was necrotic, the compacta soft and the spongiosa firm. The joint itself was not involved.

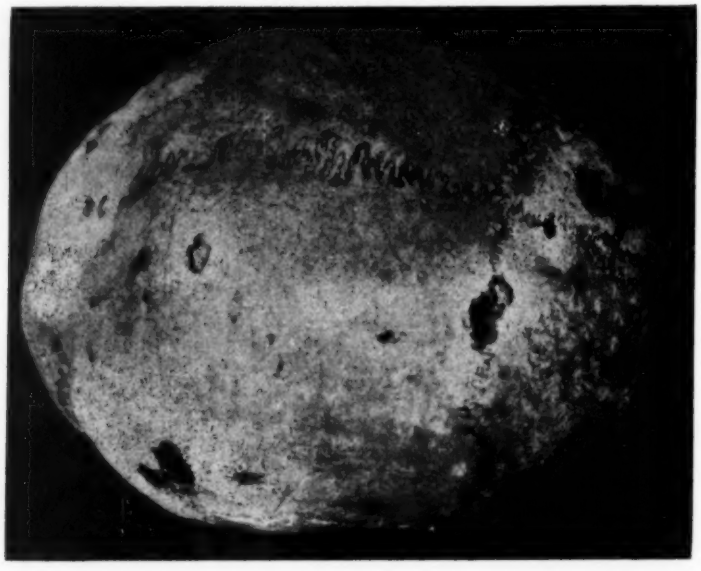

Fig. 4.—Macerated skull cap showing several osteomyelitic abscesses.

Upon removal of the skin from the left supraclavicular region a creamy gray-yellow pus escaped from a small opening in the platysma which led into the supraclavicular fossa. Upon enlarging the opening an abscess cavity was found extending upwards about 3 cm. beneath the sternocleidomastoid muscle. The periosteum of the acromial portion of the clavicle was lacking, the compacta roughened, and the surrounding structures showed no inflammatory reaction. The cavity contained about 40 c.c. of pus, with no perforation into the apex of the pleural cavity. The superficial and deep cervical, as well as the submaxillary lymph nodes were slightly enlarged, pinkish-gray, somewhat succulent, and on section were homogeneous pale gray-red.

The subcutaneous adipose tissue had almost disappeared leaving only here and there a few islands of canary-yellow fatty substance. Upon opening the pleural cavities, both lungs collapsed but were freely movable. The large vessels and the pericardial sac were covered with some fat tissue. The parietal and visceral pleura were smooth, moist and glistening. There was no

fluid in the pleural cavities. The ductus thoracicus was without change. The heart was slightly enlarged, the myocardium flabby, easily torn, and of a pale, cloudy, grayish-red color. The valves were without change.

The upper lobe of the left lung was fluffy, crepitating, slate-gray and slightly anthracotic. The lower lobe was of a semisolid consistency, non-crepitating, dark bluish-gray, somewhat anthracotic and on its anterior surface the visceral pleura was slightly thickened but smooth. On section the parenchyma of the upper lobe was gray-red, and the cut surface finely granular with a great many miliary and submiliary grayish-white nodules which were difficult to recognize, being more numerous in the upper part. The parenchyma contained air throughout and on pressure yielded a large amount of frothy bloody fluid. The mucous membrane of the large bronchi was covered with tenacious gray mucus, and the small bronchi were filled with a glassy mucus. The bronchial mucosa was gray-white, smooth, and here and there slightly congested; there was no enlargement of the bronchial lymph nodes; and on section the cut surface was smooth and uniformly deep black.

The upper and middle lobes of the right lung were slate-gray, somewhat anthracotic and slightly crepitating. The lower lobe was dark bluish-violet, and of a semisolid consistency, and upon palpation there was noted near the middle of the anterior surface just beneath the visceral pleura, a firm nodule about the size of a pea. On section the parenchyma of the upper and middle lobes was slightly congested, gray-red, its cut surface was noticeably granular and exhibited numerous gray-white miliary and submiliary nodules. The parenchyma of the lower lobe was dark brownish-red, edematous, and a bloody, frothy fluid dripped from the cut surface. Throughout the parenchyma were seen numerous gray-white miliary nodules which on section proved to be gray-yellow, dry, but without caseation. The large bronchi contained a quantity of gray, tenacious mucus; the small bronchi were also filled with a slightly bloody mucus. The mucous membrane was smooth, gray-white, here and there congested. The bronchial lymph nodes were slightly enlarged, presenting on section a smooth, deep black, cut surface. One of the lymph nodes showed numerous miliary gray-white nodules.

The pharynx, larynx, and esophagus did not show any lesions. The spleen was enlarged, fairly firm, bluish-red, with slight capsular thickening. On section the pulp was gray-red, follicles distinctly visible as grayish, opaque points and the trabeculae only visible here and there. Throughout the pulp was found many grayish-yellow, opaque, fairly well outlined nodules of a dry, cheesy consistency which measured from 1 to 3 cm. in diameter. Some of these nodules were surrounded by a small brownish-red zone.

In the right kidney was found, irregularly distributed over the cortex, many gray-white, round, indefinitely outlined areas about the size of a pinhead, which were not raised, but extended 2 to 3 mm. into the cortical substance.

After removal of the aorta and right iliac artery, there was found beneath the right psoas muscle an abscess about the size of a plum, containing a yellowish-gray, thick, mucoid material. On removing the peritoneal cover-

ing of the right side of the small pelvis and the promontorium, it was found that the abscess extended into the fifth and sixth lumbar vertebrae and into the intervertebral cartilages. The spongiosa of the anterior portion of both vertebrae was soft, pliable and could easily be removed with the knife. The whole spongiosa was bathed in a thick yellowish-gray pus.

The inguinal lymph nodes of the right side were enlarged, succulent, and on section pale gray-red.

The dura mater was thickened and showed over the right temporal area, corresponding with the above-described perforation of the right temporal

Fig. 5.—Pure culture Coccidiodes imitis from blood. Bouillon culture six weeks old.

bone, an area about 2 cm. in diameter, which was covered with a grayish red, smeary granulation tissue. The remainder of the external surface was smooth and glistening. Upon removing the brain about 50 c.c. of a slightly bloody fluid dripped off.

The brain was slightly edematous and showed many bloody points which diffused into the surrounding brain tissue. The cortex was otherwise macroscopically without change. The lateral ventricles were filled with a slightly bloody fluid; the ependyma was smooth; the choroid plexus was pinkish-gray and distinctly edematous.

MICROSCOPIC FINDINGS

Abscess of the Foot (Eosin-Hematoxylin Stain)

The epidermis was intact, but throughout the subcutaneous tissue were numerous more or less sharply outlined abscesses, and other places were diffusely infiltrated with leucocytes. Here and there were a few detached islands of epithelial cells. The leucocyte infiltrations were made up mainly of endothelial cells, polynuclear leucocytes, a few lymphocytes and plasma cells containing many cysts. Most of the cysts were surrounded by a double contoured membrane which stained either red or reddish-blue and contained either well-defined roundish bodies or honeycomb-like structures. Some of the cysts were phagocytized by large giant cells containing 15 to 20 nuclei or more, and some of the giant cells contained 2 or 3 spore-containing cysts. The lymph vessels were distinctly visible, their lumens filled with mononuclear and polymorphonuclear leucocytes. The large vessels of the subcutaneous tissue showed a marked thickening of all three coats; the walls of some of the vessels were more or less infiltrated with large and small lymphocytes and plasma cells; the areolar fatty tissue was here and there infiltrated with mononuclear cells and lymphocytes.

Lung (Eosin-Hematoxylin Stain)

Throughout the parenchyma there was numerous more or less well defined miliary and conglomerated inflammatory nodules. The majority of the areas originated in the peribronchial and perivascular connective tissue, extending into the adjacent alveoli. Others arose in the alveoli proper, being confined to one alveolus or to a group of alveoli. These areas were made up of collections of lymphocytes, lymphocytic plasma cells, endothelial leucocytes and polymorphonuclear leucocytes. The center of the nodules consisted of epithelioid cells with round or oblong nuclei and were surrounded by a small zone of homogeneous or granular protoplasm in which numerous fine fibrillae took origin. The periphery of the nodules was made up of dense masses of lymphocytes and lymphocytic plasma cells with here and there a few polynuclear leucocytes. Many of the nodules contained giant cells; the nuclei of which were either peripherally arranged or gathered near the poles. The centers of many nodules were necrotic, consisting of a reddish-stained granular débris and disintegrated nuclei. Spore-bearing and nonspore-bearing cysts in a double contoured capsule were present in the necrotic areas; some were phagocytized by giant cells. Some of the alveoli were filled with polynuclear leucocytes or with a homogeneous reddish-stained exudate. The walls of the large and small blood vessels were thickened, especially the adventitia, and the connective tissue was hyaline degenerated. The perivascular lymph spaces were here and there densely filled with carbon pigment; the stroma of the septa was thickened but not involved in the inflammatory process.

Spleen (Eosin-Hematoxylin Stain)

The splenic pulp contained numerous abscesses, some being made up entirely of polynuclear leucocytes without cysts while others contained spore-bearing or nonspore-bearing cysts and one or two giant cells. Some nodules

showed a tubercle-like structure, made up of endothelial cells surrounded by lymphocytes and a few polynuclear leucocytes. They were of two types (necrosed or nonnecrosed), both showed a few cysts and giant cells.

Fig. 6.—Pure culture Coccidiodes imitis. Agar.

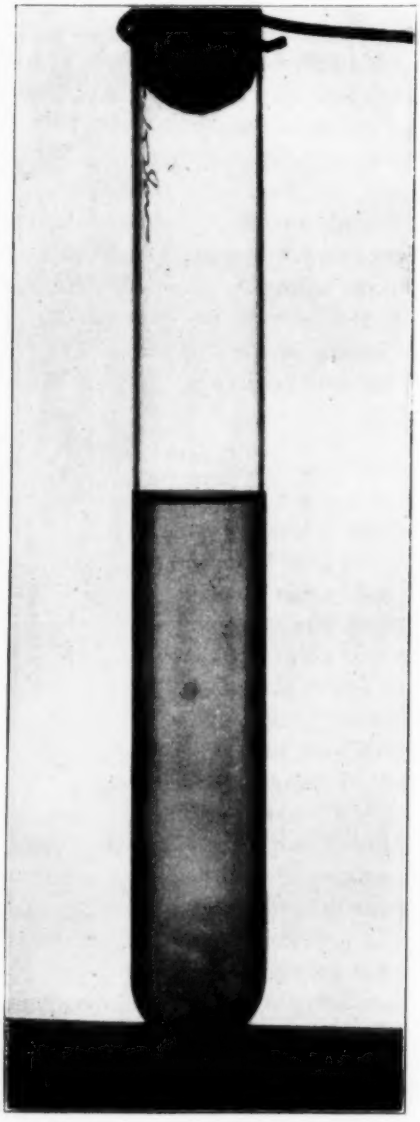

Fig. 7.—Pure culture Coccidiodes imitis. Bouillon.

Liver (Eosin-Hematoxylin and Sudan Stain)

The liver cells were extensively infiltrated with fine fat droplets, especially around the center of the acini. The remaining protoplasm was coarsely granular. The peripheral tissue showed marked round-celled infiltration and

in many places small tubercle-like formations composed of epithelioid cells and lymphocytes. Some of the tubercles contained parasites, mostly adult forms.

Kidney (Eosin-Hematoxylin Stain)

The capsule was slightly thickened and was here and there infiltrated with round cells. Throughout the cortex were many aggregations of round cells and leucocytes. The capillaries of the cortex, as well as the glomeruli, were markedly engorged with blood. The cytoplasm of the endothelial cells was distinctly vacuolated, while the nuclei were pycnotic. The capsular space was filled with a hyaline material and a few exfoliated endothelial cells. The lumen of the ascending tubules contained a fine circular reticulum.

The walls of the larger blood vessels were markedly thickened, especially the media. The adventitia was more or less sclerosed, the endothelium lining unchanged: The mucous membrane of the pelvis showed occasional infiltrations with round cells and leucocytes.

Myocardium (Sudan-Hematoxylin and Eosin-Hematoxylin Stain)

The majority of the muscle fibers contained minute fat droplets. The striations of the fibers were indistinct, the nuclei pycnotic. The interstitial tissue was markedly increased and contained large fat globules. The walls of the large and medium-sized blood vessels were greatly thickened.

Suprarenal Capsule (Sudan-Hematoxylin Stain)

The lipoid content of the cells of the fascicular zone was considerably diminished and consisted of very small fat droplets.

EXPERIMENTAL INOCULATION

Inoculation with the original pus or with a few days' old culture of the fungus in guinea pigs, white mice, and white rats showed the guinea pig to be the most susceptible. The original pus was more virulent than the cultures, since all the guinea pigs inoculated with it died; of the guinea pigs inoculated with the pure culture of the fungus only 70 per cent succumbed. White rats were refractive.

Successive inoculation from guinea pig to guinea pig increased the virulence of the fungus. Intraperitoneal inoculation killed guinea pigs in about four weeks, while subcutaneous inoculation killed them in from two to three months. If spore-bearing mycelia were injected subcutaneously, the mycelia and spores disappeared in a few days, and an abscess developed containing the typical spore-bearing cysts. From the pus of this abscess a pure culture of the fungus could be obtained.

Intraperitoneally inoculated animals showed a large caseous mass adherent to the peritoneum at the site of injection. The intestines were covered with a slimy mucoid exudate. The liver was covered with numerous small grayish-white to porcelain-white flat nodules. The lungs and spleen showed small gray-white nodules, and in both pleural cavities was a serous-fibrinous exudate. The inguinal lymph nodes were enlarged, edematous, homogeneous,

pinkish-gray, and contained a cheesy material not unlike tubercular cheese. The testicles were enlarged and infiltrated with a thick yellowish cheesy material. From all these lesions, it was possible to obtain a pure culture of the fungus. Upon sectioning the various organs, typical cysts were found.

CULTURAL FINDINGS

Cultures made from the various abscesses on plain agar incubated at 37° C. for three to four days, yielded pure colonies of a fungus-like growth, not unlike a culture of tubercle bacilli. The colonies were first round and discrete, then became more profuse in a few days, and eventually covered the entire surface of the medium.

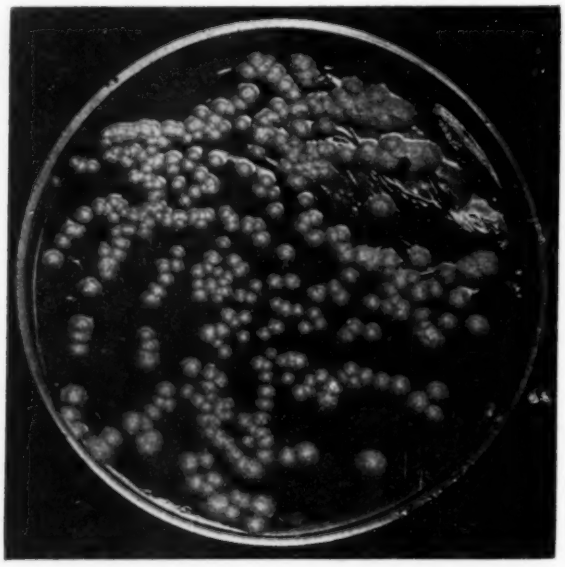

Fig. 8.—Six-day agar plate showing numerous discrete colonies.

On agar plates, containing only a few colonies, large colonies developed which measured three to four cm. in diameter and showed concentric rings not unlike Lisegang's diffusion rings. The colonies were difficult to remove from the surface of the agar because of the deep growing hyphae.

At room temperature the development of the colonies was slower, requiring six to eight days, but the aerial hyphae seemed to develop more abundantly than at 37° C. Equally good growth was observed on agar to which various sugars were added (lactose, maltose, sucrose, arabinose, L-Xylose, D-Galactose, and glucose). The most luxurious growth took place on sucrose agar.

In plain bouillon, a white flocculent growth at the bottom of the tube was noted. The supernatant liquid remained perfectly clear. Bouillon cultures three to four weeks old showed a slight brownish tinge, and in some there appeared a thick wrinkled scum on the surface. Bromcresol-purple bouillon to which the above-mentioned sugars were added showed fermenta-

tion of the sugar in each case. No gas formation was noted, while there were various degrees of acid production. Maltose and sucrose bouillon gradually turned alkaline.

Bromcresol-purple milk showed no evidence of change during the first three or four days. After six or seven days the milk turned acid and became peptonized, and at a later date it became alkaline. A light greenish scum gradually formed on the surface.

Gelatine cultures developed a surface growth similar to that occurring on agar, but was not as abundant as on the latter medium and the formation of hyphae was less pronounced. Liquefaction of the gelatine gradually occurred.

Plain or glycerinated potatoes yielded a heavy white growth with abundant aerial hyphae. No growth occurred on native decoctions of plums, raisins, or apricots.

In Fraenkel's Synthetic Medium a good growth, similar to that in bouillon, was obtained. The fungus is aerobic, no growth taking place under strict anaerobic conditions. If the cultures were protected from drying, growth continued for several months, and the cultures remained alive for six months.

Hanging drops prepared from bouillon cultures showed septated mycelia with true dichotomic branching. No spores developed in the mycelia as long as they were in the liquid medium. The aerial hyphae developed club-shaped spores as described by Ophüls. If the spores were seeded in bouillon or on agar they formed new hyphae. Potato cultures yielded an abundance of spores.

The organism was easily stained with basic aniline dyes and was gram-positive and nonacid fast.

SUMMARY

The case reported by us presents a typical infection with Coccidioides imitis of about six months' duration and with fatal termination. The initial clinical symptoms were a bronchitis with slight fever, general malaise and debility. After the temperature had subsided, pain developed in the left hip, right ankle, and thumb, simulating articular rheumatism. An abscess developed on the right side of the nose, followed by multiple abscesses of the head, thumb, and right malleolus. The abscesses were all subperiosteal, did not heal, and did not respond to any treatment. From the character and the distribution of the lesions a blood stream infection was suspected and was confirmed by a positive blood culture obtained shortly before death. The immediate cause of death was probably a toxemia.

Autopsy revealed multiple subperiosteal and osteomyelitic abscesses of the skull cap, of the second, third, and fourth metatarsal bones of the right foot, an abscess of the psoas muscle and suppurative osteomyelitis of the fourth and fifth lumbar vertebrae, subperiosteal abscess of the left clavicle extending beneath the sternocleidomastoid muscle. Miliary pneumonomycosis of both lungs, chronic bronchitis, fatty degeneration of the myocardium and chronic fibrous myocarditis, localized external suppurative pachymeningitis over the right temporal area, slight edema and hyperemia of the brain.

Smears from the various lesions showed typical spore-bearing cysts. A pure culture of the fungus could be obtained from the pus. Experimental inoculation of the pus and of the fungus in pure culture into guinea pigs produced a disease similar to that seen in man.

The case reported here undoubtedly originated in the Santa Clara Valley, since the patient had lived for the past five years in San Jose. The majority of the reported cases originated in the San Joaquin Valley, this latter locality appearing to be the main focus of the infection. The transmission of the disease is still obscure. We have no evidence that lower animals are concerned in its transmission. A saprophytic existence of the fungus in certain soils or plants is not unlikely. At present we have no specific treatment for this disease which is usually fatal.

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LABORATORY METHODS

THE LARGE MONONUCLEAR AND THE TRANSITIONAL LEUCOCYTE OF HUMAN BLOOD*

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THE two terms, "large mononuclear" and "transitional," have for many years found a place in the differential enumeration of the white cells of human blood. The two terms are now employed in a morphologic sense to designate the two types of large mononuclear cells present in normal human blood, and they have long since ceased to convey the meaning in regard to origin that Ehrlich attached to them. Briefly, the "transitional" is a leucocyte somewhat larger than a polymorphonuclear neutrophile. It has a prominent nucleus, irregular in outline and frequently indented or even horseshoe in shape. In its cytoplasm there are neutrophilic granules when stained with Wright's stain. The "large mononuclear" also is a large cell and its size is the most obvious characteristic distinguishing it from the lymphocyte. The nucleus, however, tends to be eccentric and to stain less intensely than the lymphocytic nucleus. The cytoplasm is less basophilic than the lymphocytic cytoplasm and in it there are usually a few azurophilic granules. The purpose of this paper is to describe certain microchemical and vital reactions of the "transitional" and "large mononuclear" leucocytes which are helpful in the accurate identification of them. Much of the hematologic literature is concerned with the relationship of ill-defined and more or less undifferentiated stem-cells in the adult blood-forming tissues and to an even greater extent with the embryonic origin and relationship of the blood cells. These investigations are of great interest, but the chief concern of the clinical hematologist is the accurate identification of the cell types and the significance of variations from the normal. To interpret changes in the white cells implies a knowledge of diseases affecting, directly or indirectly, the tissues that give rise to the leucocytes.

"Transitional" Leucocyte (Monocyte of Naegeli).—Naegeli[1] recognized and clearly described in the peripheral human blood a mononuclear cell that reacts with the usual peroxidase reagents. He gave it the name of monocyte. This is, of course, the cell that contains the neutrophilic granules. It is the "transitional" leucocyte. These two terms are interchangeable. Naegeli thought this cell to be of bone-marrow and splenic origin. It is incorrect and misleading to speak of the phagocytes that enter into tubercle formation, or those that are found within the sinuses of lymph nodes, as monocytes because the cells of the tubercles of human tissue and those of lymph nodes do not

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respond to the peroxidase test that colors the "transitional" leucocytes in blood smears of the same individual. The result of this simple test alone is sufficient basis for the unqualified statement that such phagocytes are not monocytes ("transitional" leucocytes). The peroxidase reaction has been subjected to criticism because the methods of using the various chemicals which have been employed to elicit it gave different results on the same tissue due to variations in the solutions used for the reaction or to the method of fixation of the tissue. However, if the reagent is applied by the same method to a given tissue the results are the same and it is in this sense that the test is significant. I[2] found that when paraffin sections of the various tissues and organs were treated with benzidin the peroxidase-reacting cells were found only in the bone-marrow, spleen, and within the blood vessels. Foci of reacting cytoplasm were observed in some of the endothelial cells lining the sinusoids of the liver, but these had the character of phagocytized material. The reticuloendothelium of the lymph nodes and many of the free cells within the sinuses did not react. Subsequently smears were made of human lymph nodes and these side by side with smears of human blood were treated with benzidin by the same technic. The lymph node is almost entirely devoid of reacting cells.[3] The "transitional" leucocyte, or monocyte, of human blood quite certainly arises from the bone-marrow and perhaps to some extent from the spleen although there appears to be no decrease in the number of this leucocyte after splenectomy.[4] The relationship between "transitional" and polymorphonuclear leucocytes with neutrophilic, eosinophilic, or basophilic granules has not been fully determined. Naegeli states that the monocyte arises from a younger cell, the monoblast, in the marrow. Although the cell has neutrophilic granules the granulation is apt to be less pronounced than that of either the neutrophilic myelocyte or the older polynuclear leucocyte. On the other hand, in the bone-marrow and in the blood of individuals with chronic myeloid leucemia there are neutrophilic myelocytes with relatively few granules and the monocyte may ultimately prove to be an older form of such myelocytes and quite closely related to the polymorphonuclear neutrophile. Not infrequently increases in "transitionals" have been observed during polynuclear leucocytoses and it should be kept constantly in mind that the "transitional" cell, or monocyte is a myeloid cell. The peroxidase* method of staining is required for the accurate identification of monocytes. Otherwise the scant number of granules present in some of these cells after polychrome staining may be overlooked, or, when present may even be confused with the azurophilic granules. In peroxidase preparations the only possibility of error is in mistaking monocytes for immature polynuclear neutrophiles. The cyto-

*A method (Jour. Am. Med. Assn., 1920, lxxiv, 17) employing benzidin for the peroxidase reaction has been in use for more than six years. It is simple and satisfactory. Smears of blood, exudates, or tissues allowed to dry in the air for a half hour, not for more than twenty-four hours, are covered for thirty seconds with the benzidin solution, which consists of 100 mg. of benzidin (dry powder) dissolved in 25 c.c. of 80 per cent pure methyl alcohol, to which 1 or 2 drops of hydrogen peroxide has been added. At the end of the half minute the alcoholic solution is diluted with an equal quantity of distilled water. The diluted reagent usually colors the granules an intense brown within three minutes. The reagent is washed off with water and the preparation blotted with blotter paper. It is now ready for the counterstain, which is made by placing Wright's stain on the preparation and at once diluting it with water. The diluted stain is allowed to act for about five minutes, since the nuclei stain less readily after the treatment with benzidin. Hematoxylin (Harris with acetic acid) diluted 1:5, applied for one minute, followed by washing and staining with eosin (0.1 per cent aqueous solution) for one minute is an excellent counterstain. The benzidin solution keeps for several months.

plasm of the latter is usually heavily studded with neutrophilic granules and the nucleus is more pyknotic.

"Large Mononuclear" Leucocytes. (Lymphendotheliocyte).—Consideration of the nonperoxidase-reacting mononuclear phagocyte of the blood is less simple. The possibility of the "transitional" leucocyte, or monocyte, being the sole mononuclear phagocyte of normal blood was considered, but I found[5] that a certain number of the mononuclear cells which do not react to benzidin ingest carbon when brought into contact with it at incubator temperature. There is therefore a nonperoxidase mononuclear cell that is phagocytic. These cells are less numerous than the monocytes and for their demonstration it is essential to collect the leucocytic layer of the citrated blood. During incubation with carbon the outlines of the ameboid cells assume a very irregular form, but in size the nonperoxidase-reacting phagocyte corresponds to the "large mononuclear" leucocyte. The combination of the peroxidase test with the phagocytic method[5] is the best one available for the positive identification of the lymphendotheliocyte ("large mononuclear" of the blood). A simpler technic is desirable.

The evidence indicates that this phagocyte is derived from the lymphoid reticuloendothelium. Sabin, Doan, and Cunningham[6] brought these cells into contact with dilute neutral red and found that a focus of dye granules (rosette) appeared in the cytoplasm at one side of the nucleus. These investigators[7] concluded that this rosette cell was derived from reticular tissue. With this I agree,[3] but in my experiments the rosette cells were found to be most numerous in the lymph nodes. Cunningham, Sabin and Doan[7] were unable to demonstrate any cells of this type is mesenteric lymph nodes. I do not, therefore, hold the same view as these investigators in regard to the distribution of the reticular tissue that gives rise to the rosette cells. Also it is essential to recognize two types of reticular tissue since the endothelium of blood capillaries may grow as reticular cells but it does not give rise to the rosette form of phagocyte.[3] Sabin, and coworkers, Cunningham and Doan, in common with a great many others speak of the rosette cells as monocytes and evidently regard them as identical with the Naegeli monocytes. The Naegeli monocyte of myeloid origin has not been demonstrated in the blood of rabbits and is present in scant numbers in the blood of normal guinea pigs. In the human being where the rosette cell (lymphendotheliocyte) is seen so clearly in tuberculous tissue and in normal lymph nodes, it may be demonstrated emphatically by peroxidase staining that the rosette phagocytes are not the Naegeli monocytes, and there is no evidence that the two types of cells are at all related.

Since the identification of the rosette cell, or lymphendotheliocyte, is dependent upon the contact of the living cell and dilute neutral red solution it is not easy to determine the complete distribution of the reticular tissue that gives rise to this type of phagocyte. By mordanting the tissue in Zenker-formol solution after injection of the dye into lymph nodes it was possible to see foci of dye granules in the larger reticular cells in the medulla of the nodes.[3, 5] Tissue cultures proved to be an effective method for bringing dilute solutions of neutral red into contact with the individual cells of the cultures. The reticuloendothelial cell (rosette) of the lymphoid tissue was found to be

almost the sole phagocyte of the lymph node cultures.[3] In tissue cultures of the spleen[9] the rosette type of phagocyte predominates and the lymphoid type of reticuloendothelium is therefore thought to be present in the spleen as a fixed tissue. The spleen is evidently only one of the contributing sources for this phagocyte, since splenectomy[4] causes no demonstrable decrease in the number of these cells appearing in experimental exudates. It has constantly been observed that this cell readily proliferates, not only in tissue cultures but also in the exudates and in various tissues where it occurs. However, it is very probable that the lymph nodes and the spleen, where these cells are present as a fixed tissue, constitute the chief source of lymphendotheliocyte supply when the demand for these leucocytes is made. In the routine examination of the lymph glands one is impressed by the great variation in the number and size of the reticular cells of the sinuses. It seems likely that the number of cells of the rosette type appearing in the blood will be found to vary with the activity of lymphoid reticuloendothelium in the lymph nodes and elsewhere.

Hemendotheliocyte.—This is the phagocyte derived from the blood vascular endothelium. The lymphoid reticuloendothelium is only one of the two types of reticular tissue. It is for this reason that it is no longer sufficient to speak of endothelial leucocytes without designating their source. There are two varieties of endothelial leucocytes. In tissue cultures of the liver of rabbits the hemendotheliocyte grows in reticular form with cytoplasmic branches connecting the individual phagocytes. When supravitally stained with neutral red it is a diffuse granule-hyaline type of cell. That is, the dye granules are scattered about diffusely in the cytoplasm. Often the dye is scant or is entirely absent with the cell appearing as an unstained hyaline cell.[3, 4, 5] This cell may appear in the peripheral blood of rabbits after the injection of large quantities of India ink.[8] There is no evidence that it is present in the normal human blood. It is almost the sole phagocyte of tissue cultures of rabbit liver.[3] In tissue cultures of rabbit spleen it is constantly present but is much less numerous than the rosette cell.[9]

Discussion.—Although the relative percentages of the granular leucocytes recorded by hematologists are about the same, one has only to consult a dozen of the current textbooks to find expression of a wide variation as regards the mononuclear leucocytes. Some workers recognize only a single group, usually either "large mononuclear" or "transitional," while others divide the leucocytes into the two groups. Within the groups, whether one or two, there are found to be variations of several hundred per cent in the figures given as the mean, minimum, and maximum for normal individuals. The explanation of this lack of standard for the mononuclear cells is that the technical methods now in common use in differential blood counting do not enable one to identify these cells accurately. The peroxidase-reacting monocyte of Naegeli, the "transitional" leucocyte, is the most numerous of the mononuclears. To determine accurately the number in human blood, the smears should be stained by a simple peroxidase method that colors only neutrophilic and eosinophilic granules. By such an examination it should be possible to establish the normal percentage for this form of myeloid cell, and finally to determine the nature and occurrence of true monocytoses.

That there is present in normal human blood a second nonlymphocytic mononuclear cell is subject to direct proof. In general structure it corresponds to the "large mononuclear." It is a nonperoxidase-reacting cell that acquires neutral red in the form of the rosette of Sabin, Doan, and Cunningham by the method of supravital staining. The evidence warrants the emphatic statement that this leucocyte is derived from reticuloendothelium of the type found in lymph nodes. Further work is required to determine fully the distribution of this type of reticular tissue. The readiness with which this cell undergoes mitosis also indicates that these leucocytes may arise in the tissues wherever they happen to be, provided they receive the proper stimulus. To identify the lymphendotheliocyte in peroxidase preparations it is necessary only to differentiate it from the larger lymphocytes. However, a method simpler than that of supravital staining, which would mark this type in a positive way, would prove very useful.

By the experimental stimulation of the blood vascular endothelium, leucocytes of hemendothelial origin can be made to appear in the peripheral blood.[5] There is no evidence that this form of leucocyte is present in normal human blood. It is a phagocyte that has comparatively little affinity for neutral red. If neutral red granules do appear in its cytoplasm they have a diffuse distribution.

CONCLUSIONS

1. The "transitional" leucocytes (monocyte of Naegeli) is the peroxidase-reacting mononuclear phagocyte of human blood. It is one of the granular leucocytes of myeloid origin. When applied as a chemical test by a prescribed technic the peroxidase reaction permits the accurate differentiation of this cell from the nonperoxidase-reacting phagocytes that have incorrectly been called monocytes.

2. The second type of mononuclear phagocyte present in the normal peripheral blood is characterized by a focus of dye granules when the living cell is brought into contact with a dilute solution of neutral red. It is a nonperoxidase-reacting cell which corresponds in its general structure to the leucocytes that have commonly been designated as "large mononuclears."

3. Under experimental conditions the third type of mononuclear phagocyte which is derived from the blood vascular endothelium may appear in the peripheral blood. By the method of supravital staining it may present a diffuse granulation or may be devoid of granules.

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A SIMPLE METHOD FOR THE DETERMINATION OF CALCIUM IN WHOLE BLOOD*

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IN THE course of our studies in calcium metabolism[1, 2] it became necessary to make determinations of calcium in whole blood as well as in serum. The following method was devised, based upon the Clark-Collip modification of the Kramer-Tisdall method for serum calcium.[3]

Principle.—The calcium is precipitated as calcium oxalate, the blood being hemolyzed by the addition of distilled water. The calcium oxalate is transformed by sulphuric acid into oxalic acid which is titrated with potassium permanganate.

Method.—Two c.c. of 4 per cent ammonium oxalate are put into an accurately graduated centrifuge tube. To this approximately 2 c.c. of blood are added, the exact amount being noted. Distilled water is immediately run in to the 15 c.c. mark. The tube is inverted a few times until the contents are thoroughly mixed. The mixture is allowed to stand for one hour and is then centrifuged at high speed for ten minutes. The supernatant fluid is poured off and the tube inverted in a rack for five minutes, the mouth of the tube resting on a pad of filter paper. The precipitate is washed once with 5 c.c. of distilled water and once with 3 c.c. of dilute ammonium hydroxide (2 c.c. of concentrated ammonium hydroxide and 98 c.c. of distilled water), centrifuging and draining each time as before described. Then 2 c.c. of approximately normal H_2SO_4 are added, being blown directly upon the precipitate to break up the mat. The tube is placed in a boiling water-bath for about one minute and the oxalic acid is titrated with 0.01 normal potassium permanganate in a water-bath at 75° C.

Calculation.—The titration value multiplied by 10, if the permanganate is exactly 0.01 normal, gives the amount of calcium in milligrams per 100 c.c. of whole blood, if the amount of blood used was 2 c.c.

Following hemolysis and centrifugation a small precipitate is thrown down with the precipitate of calcium oxalate, which we believe to consist of the stroma of the disrupted red cells. This in no way interferes with the

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reaction or titration. Varying amounts of added calcium have been successfully recovered, and the experimental error is no greater than in the original test as applied to the determination of serum calcium.

Using this method we have found the normal range of whole blood calcium to be from 6.5 to 9.5 mg. per 100 c.c., the results obtained being consistent.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LeSandier and Verge: White of Egg Culture Medium for the Gonococcus. Compt. rend. Soc. de Biol., 1925, xcii, 227.

Carefully separate the white of egg from the yolk and to one part of egg white add three parts of distilled water. Shake vigorously in a closed flask containing glass beads until the mixture becomes a homogeneous emulsion. Filter through glass wool and add 6 c.c. of glycerin to each 100 c.c. of filtrate. Sterilize for 30 minutes at 115° C. The resultant product is a viscous, slightly opalescent emulsion. One part of this emulsion is added aseptically to two parts of nutrient agar and slants prepared. On this medium the gonococcus colonies are similar to those seen on ascitic agar. All the characteristics of the organism are preserved. On this medium successful transplants have been made after 115 hours.

Burke, V., and Newton, J. L.: Preparation of Gentian Violet Solutions for Intravenous Injection. Jour. Am. Med. Assn., Feb. 20, 1926, lxxxvi, 529.

In the preparation of dye solutions for intravenous therapy the following factors must be taken into consideration: toxicity of the dye for body cells; bactericidal action in the presence of body fluids, especially blood; reaction of solution; osmotic pressure; stability and solubility of the dye in the solvent.

The authors report their studies of the most suitable solutions of gentian violet for intravenous injection and suggest that some of the uncertainty regarding the therapeutic value of gentian violet solutions may be due to variations in the solutions; as well as to variations in the defensive mechanism of the host.

The reaction of gentian violet solutions should be kept as near neutrality as possible; the more alkaline the solution the less the toxicity and the greater the bactericidal activity. A stable alkaline solution cannot, however, be prepared.

The choice of a solvent for gentian violet lies between a 3 per cent sodium bicarbonate solution and a buffered solvent.

The bicarbonate should be added after the dye is in solution and, as the solution decomposes rapidly, it must be freshly prepared and injected immediately.

A solution of 0.3 gm. of potassium dihydrogen phosphate and 0.387 gm. of dipotassium hydrogen phosphate in 100 c.c. of distilled water is a very satisfactory solvent.

The reaction is near neutrality and the solution comparatively stable.

The maximum dose of the dye will vary with the solvent and, as the toxic action is cumulative, should not be repeated too frequently.

Walker, J. E.: Effect of Mercurochrome—220 Soluble on the Germicidal Properties of Fresh Defibrinated Blood. Arch. Path. and Lab. Med., February, 1926, i, No. 2, p. 200.

Three c.c. of freshly drawn defibrinated human blood were placed in each of nine test tubes. To each tube, except the ninth which was the control and received 0.1 c.c. of normal saline, was added 0.1 c.c. of a solution of mercurochrome of such strength as to give the desired concentration in the total volume.

After mixing, 0.1 c.c. of a bacterial suspension was added. A tenth tube containing 3 c.c. of normal saline also received 0.1 c.c. of bacterial suspension.

This tube was plated at once to determine the number of organisms present and the other tubes were plated after incubation at 37° C. for varying periods (2 to 24 hours).

Mercurochrome in concentrations of 1:25,600 to 1:400 had no appreciable effect on the bactericidal activity of fresh defibrinated blood on colon bacilli; 1:200 mercurochrome destroys this activity.

Staphylococci and streptococci grow much more luxuriantly in blood containing mercurochrome 1:400 than in blood without mercurochrome, probably because of the injurious action of the dye on the leucocytes.

The use of mercurochrome in septicemia is empirical. Any beneficial results are not due to a specific action on the causative bacteria and if favorable clinical reports continue to be received, their mechanism remains a problem to be worked out.

John, H. J.: Preservation and Transportation of Blood for Chemical Study. Arch. Path. and Lab. Med., February, 1926, i, No. 2, p. 228.

Twenty mg. of a 10:1 mixture of finely powdered sodium fluoride and thymol will satisfactorily preserve 10 c.c. of blood for chemical analysis for five to seven days. As much as 150 mg. of this mixture does not alter the blood sugar.

Bunting, C. H., and Thewlis, E.: Leucocytic Reactions in Smallpox, Chicken-pox, Scarlet Fever, Measles and Mumps. Arch. Path. and Lab. Med., February, 1926, i, No. 2, 189.

Daily total and differential leucocyte counts were made in the diseases noted above. The patients were all young adults of university age, counts were all made at the same time of day (early morning) in each case.

The following classification of leucocytes was employed: neutrophils, eosinophils, basophils, small lymphocytes, large mononuclears, and transitionals.

The leucocytic pictures seen were as follows:

Scarlet Fever: A maximum neutrophilic leucocytosis occurred on the day of the appearance of the rash followed by a steady but gradual diminution of the total and neutrophilic count during the course of the disease.

Eosinophils show a rather sharp rise both in percentage and number. Basophils are uninfluenced. The lymphocytes show an early sharp percentage fall, followed by a gradual recovery reaching a peak about the end of the first week. The monocyte curve follows the lymphocyte curve.

The neutrophil curve is interpreted as a reaction to living organisms (streptococci) and the lymphocyte curve as a reaction to toxins.

Smallpox: Moderate leucopenia, usually three days before the appearance of the eruption and persisting for two to four days afterwards.

A well marked leucocytosis then occurs.

There is an early relative and absolute increase in neutrophils followed by a rapid drop below normal. When the leucocytosis develops the neutrophilic percentage remains low but the total number remains near normal.

Eosinophils are low; basophils show no striking change. An early reduction in the lymphocytes is followed by a sharp rise with great variation in the size of the cells.

In early cases certain bodies—classified as "Councilman bodies" were seen in the large lymphocytes: one type was protoplasmic, about the size of the nucleus, staining a light clear blue with Wright's stain; others seemed to be small reddish (metachromatic) granules often surrounded by an apparent vacuole and seen in the nuclei. They are believed to be peculiar to the disease.

Chicken-pox: Qualitatively the picture follows that of smallpox; quantitatively the range of cellular variation is smaller.

Measles: Primary leucopenia with a percentage rise in neutrophils, followed by a rapid decrease. The basophils show no marked change but there is a tendency toward a moderate eosinophilia. There is an initial lymphopenia influenced by the intensity of the infection.

Mumps: Moderate leucopenia at the onset followed by a gradual return to normal and thence to a moderate leucocytosis. An early percentage increase of neutrophils is followed by a gradual decrease. After an early deficiency there is a percentage and total increase of eosinophils. An early lymphopenia is followed by a lymphocytosis.

The pictures seen and their interpretation are discussed. They have no diagnostic value but, in conjunction with clinical findings, may prove suggestive.

Castellani, A., and Taylor, F. E.: Fallacies of the "Yeast Method" of Detecting Glucose in the Urine. Jour. Am. Med. Assn., Feb. 20, 1926, lxxxvi, No. 8, p. 524.

Contrary to long-held opinion, the fermentation test with yeast is not a specific or infallible test for glucose in the urine.

The fallacy was first reported by Castellani in 1904 and confirmed by Castellani and Taylor and others and attention is again called to this misconception in the present paper.

Ordinary baker's yeast, as usually obtained, contains more than one species of yeast and almost constantly a gram-positive bacillus, and such preparations will ferment almost any sugar. Even with pure yeast cultures the authors obtained fermentation of levulose, galactose, saccharose, and maltose as well as glucose. Fermentation in the urine, therefore, is not conclusive evidence of the presence of glucose.

Organisms fermenting glucose only are extremely rare. One of the few such organisms is M. balcanica. The next most suitable monilia which ferments, with the production of gas, only glucose and levulose, is Monilia krusei, Castellani.

The table below shows the fermentative characters of certain monilias and bacteria.

By using the following mycologic formula the identification of reducing substances may be attained.

The substance to be studied is made in a 1 per cent solution in sugar-free peptone water, distributed in Durham fermentation tubes, and the tubes are inoculated with various monilia in accordance with the suspected nature of the substance.

In testing urine, the specimen must be collected aseptically or sterilized in the Arnold for 30 minutes. Autoclaving alters sugars and so is not permissible. If desired the specimen may be passed through a Chamberland or Berkefeld filter. About one-third the volume of peptone water is added to encourage growth.

The urine is distributed into six tubes, each containing a Durham's fermentation tube. They are labeled 1, 2, 3, 4, 5 and 6. Tube 1 should be inoculated with Monilia balcanica Castellani, or M. parabalcanica Castellani; tube 2 with M. krusei Castellani; tube 3 with M. pinoyi Castellani; tube 4 with M. metalondinensis Castellani; tube 5 with B. coli-communior Escherick, and tube 6 with B. paratyphosus B Schottmüller.

After the tubes are incubated at 35 C. for four days, the results may be read:

1. If in tubes 1, 2, 3, 4, 5 and 6 gas is present, the reducing substance is glucose.

2. If tube 1 contains no gas, and in tubes 2, 3, 4, 5 and 6 gas is present, the reducing substance is levulose.

3. If tubes 1 and 2 contain no gas, and in tubes 3, 4, 5 and 6 gas is present, the reducing substance is maltose.

4. If tubes 1, 2 and 3 contain no gas, and in tubes 4, 5 and 6 gas is present, the reducing substance is galactose.

5. If tubes 1, 2, 3 and 4 contain no gas, and in tubes 5 and 6 gas is present, the reducing substance is a pentose.

6. If tubes 1, 2, 3, 4 and 6 contain no gas, and in tube 5 gas is present, the reducing substance is lactose.

7. If none of the tubes contain gas, the reducing substance belongs to the non-fermentable group of reducing substances, including uric acid, creatinine, hippuric acid, and others.

TABLE I

FERMENTATIVE CHARACTERS OF CERTAIN MONILIAS AND BACTERIA*

	Glucose	Levulose	Maltose	Galactose	Saccharose	Lactose	Mannitol	Dulcitol	Dextrin	Raffinose	Arabinose	Adonitol	Inulin	Sorbitol	Starch	Glycerol	Inositol	Salacine	Amygdalin	Isodulcitol	Erythrotol
Monilia balcanica (Castellani)	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia krusei (Cast.)	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia pinoyi (Cast.)	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia metalondinensis (Cast.)	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia tropicalis (Cast.)	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia rhoi (Cast.)	+	+	0	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia bronchialis (Cast.)	+	+	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monilia macedoniensis (M. macedoniensoides) (Cast.)	+	+	0	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0
Bacillus coli-communis (Esch.) strain 0	+	+	+	+	0	+	+	+	+	+	+	0	0	+	0	+	0	+	0	+	0
Bacillus coli-communior, strain 0	+	+	+	+	+	+	+	+	+	+	+	0	0	+	0	+	0	+	0	+	0
Bacillus paratyphosus B. var. M. Schottmüller	+	+	+	+	0	0	+	+	+	0	+	0	0	+	0	0	+	0	0	-	0
Bacillus paratyphosus A. (Schott.)	+	+	+	+	0	0	+	+	+	0	+	0	0	+	0	0	0	0	0	-	0
Bacillus columbensis (Cast.)	+	+	+	+	0	0	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0
Bacillus asiaticus (Cast.)	+	+	+	+	+	0	+	0	+	+	+	0	0	+	0	+	0	0	0	+	0
Bacillus pseudoasiaticus (Cast.)	+	+	+	+	+	0	+	+	+	+	+	0	0	+	0	+	0	+	0	+	0
Bacillus morgani (Cast. and Chalm.), strain S	+	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*In the tables and in the mycologic formulas, + indicates gas; 0, the absence of gas; simple acid fermentation is not taken into account.

MYCOLOGIC FORMULAS

Glucose

Monilia balcanica (Cast.)	+	=	Glucose.

Levulose

Monilia krusei (Cast.)	+	=	Levulose.
Monilia balcanica (Cast.)	0		

Maltose

Monilia tropicalis (Cast.)	+	=	Maltose.
Monilia macedoniensis (Monilia macedoniensoides), (Cast.)	0		
Monilia metalondinensis (Cast.)	+	=	Maltose.
Monilia macedoniensis (Cast.) or (Monilia macedoniensoides)	0		
Monilia pinoyi (Cast.)	+	=	Maltose.
Monilia krusei (Cast.)	0		
Monilia pinoyi (Cast.)	+	=	Maltose.
Monilia macedoniensis (Monilia macedoniensoides), (Cast.)	0		
Monilia tropicalis (Cast.)	+	=	Maltose.
Monilia rhoi (Cast.)	0		
Monilia pinoyi (Cast.)	+	=	Maltose.
Bacillus morgani (Cast. and Chalm.)	0		
Monilia metalondinensis (Cast.)	+	=	Maltose.
Bacillus morgani (Cast. and Chalm.)	0		

Galactose

Monilia metalondinensis (Cast.)	+	=	Galactose.
Monilia pinoyi (Cast.)	0		
Monilia metalondinensis (Cast.)	+	=	Galactose.
Monilia krusei (Cast.)	0		
Monilia macedoniensis (Cast.)	+	=	Galactose.
Monilia tropicalis (Cast.)	+		
Monilia bronchialis (Cast.)	0		
Monilia tropicalis (Cast.)	+	=	Galactose.
Monilia macedoniensis (Cast.)	+		
Monilia krusei (Cast.)	0		
Bacillus paratyphosus B. (Schott.)	+	=	Galactose.
Bacillus morgani (Cast. and Chal.)	+		
Monilia krusei (Cast.)	0		

Saccharose

Monilia tropicalis (Cast.)	+	=	Saccharose.
Monilia metalondinensis (Cast.)	0		
Monilia tropicalis (Cast.)	+	=	Saccharose.
Bacillus coli-communis, sensu stricto (does not ferment saccharose), (Escher.)	0		
Monilia tropicalis (Cast.)	+	=	Saccharose.
Bacillus paratyphosus B (Schott.)	0		
Monilia macedoniensis (Cast.)	+	=	Saccharose.
Bacillus coli-communis, sensu stricto (Escher.)	0		
Bacillus coli-communior	+		
Monilia macedoniensis (Cast.)	+	=	Saccharose.
Bacillus paratyphosus B (Schott.)	0		
Bacillus coli-communior	+		
Bacillus coli-communis, sensu stricto (Escher.)	0	=	Saccharose.
Bacillus neapolitanus (Emmerich)	+		
Bacillus coli-communis, sensu stricto (Escher.)	0	=	Saccharose.
Bacillus asiaticus (Cast.)	+		

Inulin

Monilia macedoniensis (Monilia macedoniensoides), (Cast.)	+	= Inulin.
Monilia tropicalis (Cast.)	0	
Monilia macedoniensis (Cast.), (Monilia macedoniensoides)	+	= Inulin.
Monilia rhoi (Cast.)	0	
Monilia macedoniensis (Monilia macedoniensoides), (Cast.)	+	= Inulin.
Bacillus coli-communior (Escher.), Bacillus pseudocoli (Cast.), Bacillus neapolitanus (Emm.)	0	
Monilia macedoniensis (Monilia macedoniensoides), (Cast.)	+	= Inulin.
Bacillus asiaticus (Cast.)	0	

Inositol

Bacillus paratyphosus B, Var M (Schott.)	+	= Inositol.*
Bacillus paratyphosus A (Schott.)	0	

Glycerol

Bacillus asiaticus (Cast.)	+	= Glycerol.*
Bacillus paratyphosus B, Var M (Schott.)	0	
Bacillus columbensis, Strain L (Cast.)	+	

CHEMICOMYCOLOGIC FORMULAS

Saccharose

Fehling	0	= Saccharose.
Monilia tropicalis (Cast.)	+	

Lactose

Fehling	+	= Lactose.
Bacillus paratyphosus B (Schott.)	00	
Bacillus coli-communis (Esch.)	+	

Pentose

Fehling	+	= Pentose.*
Monilia tropicalis (Cast.)	0	
Bacillus paratyphosus B (Schott.)	+	
B. Coli-communis (Esch.)	+	

*With great probability.
*Generally arabinose.

Keiding, E., and Keiding, T.: Method for the Demonstration of Small Quantities of Gold in Organic Substances. Acta Tub. Scand., 1925, i, 200.

The method described has been tested in 2000 cases and is claimed to be sensitive to 0.001 mg.

Destruction of organic material: Accomplished by evaporation and incineration of finely divided organs, fecal matter, blood, or urine.

The ashes of the urine, organs, blood, and feces are dissolved by boiling three times with 10 c.c. of aqua regia and pouring all the solutions into one beaker. The mixture is then diluted to 200 c.c. with water and evaporated to one-fourth its volume.

After cooling, 50 c.c. of a 50 per cent solution of potassium carbonate and 25 c.c. of an 8 per cent solution of sodium hydroxide are added and, shortly after, 50 c.c. of 5 per cent of sodium sulfite.

Mix, heat to boiling, cool and filter.

Acidulate the filtrate with concentrated HCl, add 0.5 gm. of potassium chlorate and boil for a few minutes.

Precipitation of the gold:

The solutions of organic ashes or urine thus obtained are diluted with water to 250 c.c. in a 2 liter beaker and 200 c.c. of 8 per cent sodium hydroxide and 100 c.c. of 1 per cent magnesium sulfate are added.

The beaker is placed in a water-bath at 65° C. and while stirring rapidly, 50 c.c. of 30 per cent hydrogen peroxide is added. The temperature is maintained at 65° C. for 2 hours and must not vary from 60° to 68° C. With a gold content of 0.5 mg. or over, the addition of the hydrogen peroxide is at once followed by a red coloration of the magnesium hydroxide in the fluid. When small quantities—0.1 to 0.2 mg.—are present, the color can be seen only when the precipitate has settled.

After two hours on the water-bath the beaker is placed over the open flame and heated until all the hydrogen peroxide is boiled out (moderate boiling without violent bubbling).

Dilute with boiling water to 2 liters and allow to stand until the flocculent precipitate of magnesium hydroxide and gold has settled out. The color of the precipitate depends upon the gold content.

Siphon off the supernatant fluid and make up the volume of deposit to 100 c.c.

Compare with standard gold solutions similarly treated.

Thomson, D., and Thomson, R.: The Preparation of High-Class Nutrient Media for the Cultivation of Germs Which Are Very Difficult to Grow. Ann. Pickett-Thomson Research Lab., June, 1925, i, 217.

The methods reported were elaborated during an investigation to find media suitable for the massive growth of gonococci for the preparation of large amounts of gonococcus vaccine during the World War.

It was found that the prolonged heating required in the usual method of preparing agar media produced an agar by-product directly inhibitory to bacterial growth. The methods described are rapid and avoid overheating.

Methods.—1. Preparation of agar solution: agar fiber, sufficient to make a 5 per cent solution, is weighed and cut into short lengths. Add tap water and bring to a boiling point over a gas ring. The material must be constantly stirred to prevent burning. The resultant solution is invariably neutral.

2. Preparation of testicular bouillon with peptone and salts.

Ox testicles from the slaughter house are separated from their fibrous covering and minced as finely as possible in a meat grinder. The resultant fluid mass is weighed, an equal weight of tap water added, and the mixture slowly heated in an open vessel to the boiling point.

The heating should take from 20-30 minutes. When the boiling point is reached, quickly separate the coagulum by straining through a cloth. The resultant infusion is a pale, yellowish, milky fluid. It can be used at once or, after sterilization at 116° C. in the autoclave, may be stored.

If stored and to be used for liquid media, the precipitate which forms should be removed by adjusting the reaction to P_H 4.5, heating to 60° C. and filtering, or, preferably, by centrifuging in a Sharples centrifuge. If intended for a solid medium, removal of the precipitate is not necessary.

The clear fluid so obtained is brought to a P_H of 8.4 and the resultant precipitation of phosphates removed. After neutralization it may be used to prepare bouillon.

To a quantity of this testicular infusion add 4 per cent of peptone and 0.6 per cent of sodium dihydrogen phosphate. Ringer's salts may be used instead, if desired, the formula following:

Sodium chloride	9.0 gm.
Calcium chloride	0.25 gm.
Potassium chloride	0.42 gm.
Sodium carbonate	0.3 gm.
Distilled water	1000 c.c.

Ringer's solution does not appear to have any advantage over disodium hydrogen phosphate.

The peptone and salts are dissolved over the open flame by slow heating continued until the boiling point is reached.

Reaction: adjusted to P_H 7.7 in a comparator by using phenolsulphonepthalein as an indicator. After autoclaving for 30 minutes at 116° C. the reaction will be decreased by P_H 0.2. This material is then added to the agar solution.

Rapid Process of Combined Sterilization, Clarification, and Sedimentation: The apparatus, devised by Mr. Downing, is constructed as follows:

A metal can of one or two gallons capacity is secured, consisting of a cylindrical body, a conical shoulder, and a cylindrical neck capable of holding a rubber stopper about 1¼ inches in diameter.

This stopper has two perforations in which are fitted two glass tubes, one reaching almost to the bottom of the vessel and serving as an air inlet, the other just long enough to reach above the layer of sediment which settles in the conical shoulder of the vessel when inverted. The length of this tube will naturally depend upon the shape and size of the can used. It provides outflow for the medium and is attached by a short length of rubber tubing to a hooded outlet. The flow is controlled by a Mohr clip.

To the air inlet tube a short glass tube plugged with cotton is attached by a short rubber tube and serves as an air filter.

The agar medium to be filtered is placed in the can which is plugged with cotton and placed in the autoclave. The rubber stopper with its attachments is wrapped in a towel and also placed in the autoclave.

A temperature of 116° C. is maintained for 30 minutes when all are at once removed.

Sufficient 20 per cent sterile solution of glucose is now added to the medium to make a concentration of 0.5 per cent.

The stopper is inserted, securely tied in and clips are attached to both rubber tubes.

The apparatus is then inverted and placed in a retort stand. The air filter, previously sterilized in the hot air oven is attached, the clip being removed. The entire apparatus is then placed in the Arnold adjusted to 60° C. for 30 minutes to allow sedimentation to occur.

After the medium is thus clarified by sedimentation, the apparatus is removed, a new sterile air filter attached, and the medium tubed or flasked.

The first few centimeters may be cloudy and are discarded. The remainder will be perfectly clear.

Enriching Fluids.—The most suitable are human body fluids such as whole blood, blood plasma, blood serum, hydrocele fluid, pleuritic fluid, and ascitic fluid.

Blood is secured from patients requiring the Wassermann test and is kept from clotting by the addition of a small amount of 5 per cent sodium citrate. Either the whole citrated blood or the clear supernatant plasma may be used.

Blood clots from Wassermann specimens may also be used, the clots being broken up with glass beads and the resultant fluid boiled to sterilize it. Boiled blood is a very valuable enriching substance in the experience of the authors. If there is any doubt as to the sterility of the blood they heat it for one hour at 56° C.

Blood clots in the initial stages of decomposition may be used, and, indeed, this medium containing the products of decomposition seems to possess certain advantages for bacteria requiring hemoglobin.

Hydrocele fluid is filtered through paper if a coagulum is present, the reaction adjusted to P_H 7.5, and a small quantity of Kieselguhr added. It is then filtered through a Berkefeld candle into sterile flasks for storage.

Addition of Enriching Substances.—A suitable amount—10 per cent—is placed in the sterile tubes or flasks and sterile agar added directly from the sedimenting vessel. For bacteria requiring hemoglobin the tubes or flasks are boiled for one minute and then cooled. This permits the use of blood which has become slightly septic. By the use of such media many organisms have been isolated from sputum, apparently for the first time.

Fluid Media.—The advantages of the enriching substances described may be secured in fluid media by the following procedure: The enrichment substances are added to agar as a slant culture and the tube is then filled with testicular bouillon. The growth-stimulating substances enter the bouillon by diffusion.

To obtain maximum growth the reaction must be kept neutral. Phenolsulphonephthalein is therefore added to the liquid medium and, as required, sufficient sterile sodium hydrate solution is added to bring out the pink color.

It is suggested that the media described possess many advantages over those in routine use and should be on hand for everyday purposes.

Cantero, A.: Bacteriology of the Thyroid Gland in Goiter. Surg., Gynec., and Obst., January, 1926, p. 61.

Cantero reports a bacteriologic study of 50 goiters, mainly of colloid and adenomatous types. Growth was obtained from all but three of the specimens.

The tissues were examined immediately after removal. After searing, a small portion was excised, washed in sterile N/S, emulsified in N/S and inoculated into various media including glucose-brain-broth and glucose-brain-agar. Anerobic cultures were also made.

The brain broth was made from Difco dehydrated broth to which was added 0.2 per cent glucose, about 2 gm. of calf's brain and several small pieces of marble.

All media were adjusted to P_H 6.8 to 7.2, sterilized at 20 pounds for 20 minutes, and clarified by a continous feed centrifuge.

Cultures were incubated at 37° C. for 7 days and examined daily.

Organisms belonging to the streptococcus group were isolated in 31 cases; pneumococci in 2; Welch's bacillus in 2; staphylococci in 7 cases; a diphtheroid, B. pyocyaneus, and M. tetragenus were each found once.

The streptococci were of both hemolytic and viridans type.

It is suggested that the localization of streptococci may be a factor in the pathogenesis of goiter.

Burgess, J. F.: On Some Aspects of the Cultural Study of the Ringworm Fungi. Canadian Med. Assn. Jour., 1925, xv, 1003.

By the microscopic examination of scales or hair stumps treated with 15 per cent potassium hydroxide the fungi may be classified as:

1. Microsporon: round spores 3 to 4 micra in diameter arranged in a mosaic around the affected hair stump.

2. Trichophyton: spores 3 to 4 to 5-7 micra in diameter, oval or oblong, and arranged in chains.

3. Epidermophyton, found only in scales and seen as wavy, segmented strands.

4. Achorion: irregularly segmented spore-like elements seen in material obtained from the characteristic cup-like lesions.

Material for culture, if moist, is allowed to dry in sterile tubes for four to five days before culture.

Hair stumps or scales are cut into small pieces with a sterile razor on a sterile slide and the pieces—5 to 6 to each tube— planted on 6 per cent glycerin agar. Incubation is at room temperature and, while growth usually appears in 6 to 20 days, the rarer forms may require a period of 2 months.

The morphology of cultures varies greatly from that of the fungus seen in lesions.

It is necessary, for classification, to use a standard medium made with imported peptone.

The formula for Sabourand's "media d'epreuve" is:

Glucose C. P.	4 gm.
French peptone (Chassaing)	1 gm.
Agar	3 gm.
Distilled water	100 c.c.

The imported peptone may be secured from the E. P. Dolby Co., Philadelphia, Penn.

The "media de conservation" has the following formula:

Peptone (Chassaing)	3 gm.
Agar	2.5 gm.
Distilled water	100 c.c.

A large surface for growth is necessary, and plants are made on the surface furnished by 60 c.c. of agar in 300 c.c. Erlenmeyer flasks.

Pleomorphism after culture is common and transplants always yield pleomorphic varieties.

Pleomorphism may be prevented by the use of the "media de conservation" after isolation.

For microscopic study of cultures hanging-drop preparations are prepared as follows:

An ordinary rubber washer is sterilized by boiling and fixed to a sterile glass slide by sterile vaseline. A drop of 4 per cent glucose broth is placed in the center of another sterile slide, inoculated with a small portion of the culture, and the slide inverted over the washer the edges of which have been smeared with sterile vaseline. This forms an air-tight chamber. From 15 to 20 such preparations should be made to compensate for failure of growth, evaporation, etc.

The guinea pig is suitable for inoculation on partially denuded areas, lesions appearing in 7 to 12 days and disappearing spontaneously in 30 to 40 days.

The article is illustrated.

Kline, B. S., and Young, A. M.: A Microscopic Slide Precipitation Test for Syphilis. Jour. Am. Med. Assn., March 27, 1926, lxxxvi, 928.

The technic is as follows:

Glassware and Apparatus—Ordinary microscopic slides are washed in soap and water, rinsed thoroughly in water, allowed to remain in 95 per cent alcohol for a short time, dried and then flamed. After this, four paraffin rings (each with an inside diameter of 11 to 12 mm.) are made on one surface according to the method of Green[1] by transferring a small amount of hot paraffin on a stiff wire (gage 19) wound with thread (or hat wire) bent to the form of a circle.

The pipettes needed for delivering the serums are the ordinary 1 c.c. pipettes graduated in 0.01 c.c. The pipettes for the antigen are the same as those for the serums with the ends drawn out so that each drop of antigen equals 0.015 c.c. The diameter of the tip over all is about 1.25 mm.

Vials for preparing the antigen dilution are similar to those used and recommended by Kahn.

A humidor cover is necessary for the test. The one employed in this laboratory consists of a wooden lid, 16½ by 4 by 1½ inches inside diameter, with a moistened blotter fastened in place with thumb tacks.

Antigen.—The antigen and antigen dilution are prepared as for the Kahn test. The antigen citration likewise is done as for the Kahn test. The antigen dilution should be made up just before pipetting the serums. Some antigen dilutions have been found to work only within fifteen minutes of their preparation. An average antigen dilution may still be used forty-five minutes after its preparation. The action of the antigen dilution has been found unsatisfactory when the room temperature and that of the microscopic slides is low; again, false clumping occurs in serum antigen dilution mixtures allowed to dry. Accordingly it is important to do the test in a warm, humid room (about 80° F. with visible moisture on the windows), which is readily prepared by heating a pan of water after closing the windows and doors. The table top on which the tests are performed should be kept warm. A piece of harness felt three-fourths inch thick is satisfactory for this purpose.

Serums.—These are obtained as for the Wassermann test, care being exercised that they contain no red blood cells or foreign material. Before use, they are heated to 56° C. for one-half hour.

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[1]Green, G.: Am. Jour. Publ. Health, 1925, xv, 651.

The test.—Into each ring, 0.06 c.c. of the undiluted serum to be tested is delivered from a pipette. It is advisable to work with not more than twelve serums (three slides) at a time. After all the serums are pipetted, 1 drop of Kahn's antigen dilution (0.015 c.c.) is allowed to fall into the serum in each ring. After all the antigen is pipetted, the small amount in each ring is evenly distributed by rapidly stirring the mixture with a toothpick (a new toothpick with flat end a few millimeters in width is used for each test). After the mixtures are complete, the slides, without further agitation, are placed below the humidor cover and allowed to remain at room temperature for ten minutes. At the expiration of this time, the first slide is removed, rocked and rotated by hand for about sixty seconds (about sixty times) and read immediately. The readings are made through the microscope (16 mm. objective, 10 or 12.5 eyepiece) with the light cut down as in studying urinary sediments and recorded in terms of plusses according to the size of the clumps. Because of the importance of the test it is strongly recommended that it be done in duplicate, different antigens being used.

In performing the tests, capillary pipettes (drawn from glass tubing) with bulbs holding about 10 drops of fluid may be used instead of those described.

Bauer, E. L., and Wilmer, H. B.: Toxin-Antitoxin. Hypersensitivity to Its Protein Content. Jour. Am. Med. Assn., March 27, 1926, lxxxvi, 942.

A five year clinical study has convinced the authors that toxin-antitoxin, the protein of which has been removed by the methods of Park and Banzhof, does not produce hypersensitivity to horse protein. This product, therefore, is not contraindicated in asthmatic patients.

Saunders, A. P.: Meinecke Microreaction in Syphilis. Arch. Path. and Lab. Med., March, 1926, i, No. 3, p. 388.

Method.—The blood can be obtained from the ear, one drop being drawn up into a tube 10 cm. in length and 1 to 2 mm. in width. Then one end of the tube is sealed if the examination is to be made in the same place, otherwise both ends need to be sealed. After coagulation has taken place, this tube is centrifuged and the part of the tube containing the clear serum is separated from the rest by means of a glass file and is then ready for use.

To 600 c.c. of a 3 per cent solution of sodium chloride 0.1 gm. of sodium carbonate is added. Of this dilution 5 c.c. are measured into one test tube and 0.5 c.c. of the Meinicke extract into a second test tube. Both tubes are put into a water-bath of 45° C. for ten minutes. The warm extract and the sodium solution are mixed quickly. A cover glass preparation is now made, using one small platinum loop full of blood serum to one large platinum loop full of diluted extract. The large platinum loop must be of such a size as to hold exactly five times as much as the small loop. Serum and extract are thoroughly mixed on the cover glass, and a hanging-drop preparation is made. The slide is kept in the dark at room temperature of not more than 20° C. for one hour, when the reading can be made under the microscope with a high dry lens. In negative specimens the field looks almost empty; here and there a few dancing spots are seen. In positive specimens definite clumping has taken place, giving a similar picture to the positive Widal reaction. Instead of the platinum loop method, which may become inaccurate easily, leucocyte pipettes have been used.

Meinicke recommends not to make more than ten preparations with the same extract dilution, as on long standing an opacity may be produced giving a false positive reaction.

The reading should be made at the end of one hour; a second reading may be made after another hour, but a longer delay is likely to cause unspecific changes which render the results a good deal more indefinite and even false.

Huddleson, I. F.: Is Bacterium Abortus Pathogenic for Human Beings? Jour. Am. Med. Assn., March 27, 1926, lxxxvi, 943.

Two cases are reported in which infection probably occurred from infected milk.

Szues, E.: Sputum Examination in So-Called Closed Tuberculosis. Jour. Am. Med. Assn., March 27, 1926, lxxxvi, 946.

The following method was successful in demonstrating tubercle bacilli in so-called closed tuberculosis without expectoration: A cotton plug wrapped around a throat applicator is introduced as far as the epiglottis and the patient ordered to cough. A smear is made from the almost invisible material thus obtained.

Grove, J. S., and Kramer, S. E.: Primary Carcinoma of the Lung. Am. Jour. Med. Sc., February, 1926, clxxi, 250.

A clinical and pathologic study of 21 necropsies and 3 biopsies with a review of the literature and bibliography. A valuable paper as a reference on this subject.

Larson, W. P., and Eder, H.: Immunization Against Diphtheria with Toxin Detoxified with Sodium Ricinoleate. Jour. Am. Med. Assn., April 3, 1926, lxxxvi, 998.

The authors report the clinical application of soap-detoxified diphtheria toxin experimentally devised by Larson, to the immunization of the human being against diphtheria. The method of detoxification has been described previously and abstracted in this Journal.

In 576 cases immunization was successful without the production of reactions; more rapid than by the usual method; and without sensitization of the patient.

Larson, W. P., Huenekens, E. J., and Calby, W.: Immunization Against Scarlet Fever with Toxin Detoxified with Sodium Ricinoleate. Jour. Am. Med. Assn., April 3, 1926, lxxxvi, 1000.

Sodium ricinoleate is an effective agent in detoxifying scarlet fever streptococcic toxins and such soap-toxin mixtures give a rapid immunity without causing objectionable reactions. Sufficient detoxified toxin can be given in a single injection to cause positive skin tests to become negative in a few days, 77.3 per cent of 1,542 cases became negative in eight days and 97 per cent in three weeks.

The authors believe that the antigen should contain not only the scarlet fever toxin but the streptococci as well in order to develop an antibacterial as well as an antitoxic immunity and so provide a double security.

Cherry, T. H.: The Relation of Blood Sedimentation to Pelvic Disorders. Am. Jour. Obst. and Gynec., January, 1926, xi, 1.

After a study of this test in 71 operative cases of adnexal disease the following conclusions were reached:

The rate of sedimentation is easily influenced in diseases by absorption into the general circulation of unknown substances that produce a change in the chemical and physical balance of the blood elements.

The increased sedimentation rate does not, however, indicate the degree and virulence of the infection; neither, from the observations made, does a lower sedimentation time signify a bad prognosis. A *steady* lowering of the time means, however, an increasing toxemia.

As a diagnostic agent alone, the test is untrustworthy in pelvic conditions.

In pelvic infections the leucocyte count is a more reliable indicator of the degree and virulence of the infecting organism.

Hinkleman, A. J.: The Differential Count in Acute Surgical Conditions. Jour. Kans. Med. Soc., November, 1925, xxv, 1.

Hinkleman draws the following inferences from the differential leucocyte count.

The diseases and infectious processes which, when uncomplicated, consistently give rise to a leucocytosis in which the polymorphonuclear cells alone are increased are: acute

appendicitis, peritonitis, strangulated hernia, intestinal obstruction, infection of the uterine cavity, ruptured ectopic, eclampsia, tetanus, and acute acidosis from any cause.

Those diseases producing a leucocytosis in which all the various types of leucocytes are more or less increased include the following: acute salpingitis and infection of the ovary, pyelitis, cystitis, infection of the prostate gland and other organs or parts of the male genitourinary tract, hepatic colic, and practically all acute infections of tissues outside of the abdominal cavity and not mentioned in the previous classification.

Ecker, E. E., and Megrail, E.: Production of Toxic Substances in Young Cultures of Single Cell Strains of B. Paratyphosus B. Jour. Infect. Dis., December, 1925, xxxvii, No. 6, 546.

Berkefeld N filtrates of 2 per cent Witte peptone veal infusion broth cultures of 5 single cell strains from each of two cultures of B. paratyphosus B. injected intravenously into rabbits proved to be as toxic as the filtrates of parent cultures. Boiling for three minutes did not lessen the toxicity. No significant cultural differences were noted between the single cell and parent strains.

Brown, H. C., Duncan, J. T., and Henry, T. A.: The Differentiation of Food-Poisoning Bacteria. The Lancet, London, Jan. 16, 1926, p. 117.

The salts below are suggested as an additional means of differentiating organisms of the Salmonella group of bacteria.

Peptone water containing one per cent of the sodium salts of citric, d-tartaric, l-tartaric, m-tartaric, fumaric and mucic acids, is used as the culture medium.

After incubation of the cultures for 18 to 96 hours 0.6 c.c. of a saturated solution of lead acetate is added for each 5 c.c. of the tartrate or citrate medium.

All of the acids yield insoluble lead salts and decomposition of the acid salts is evidenced by a decreased precipitation.

Incubation of the fumaric acid medium should be continued for 96 hours as decomposition is slow.

With mucic acid a little acetic acid should be added to dissolve lead carbonate which may form and so distinguish it from lead mucate.

The reactions obtained are indicated below:

	CITRATE	D-TARTRATE	l-TARTRATE	M-TARTRATE	FUMARCITE	MURATE
B para A	−	−	−	−	−	−
B para B	+	−	+	−	−	+
B para C	+	+	−	+	−	−
B. Suipestifer	+	+	−	+	+	−
Salmonella type G	+	+	−	+	+	−
Type Reading	+	+	+	+	+	+
Type Mutten	+	+	+	+	+	+
Type Newport	+	+	+	+	+	+
Type Binns	+	+	+	+	+	+
Type Derby	+	+	+	+	−	+
B. Gaertner	+	±	±	±	+	+

Rockwell, G. E., and Highberger, J. H.: Carbon Dioxide as a Factor in the Growth of the Tubercle Bacillus and of Other Acid Fast Organisms. Jour. Infect. Dis., January, 1926, xxxviii, 92.

It is shown that the inhibition of growth of a saphrophytic tubercle bacillus, two strains of virulent tubercle bacilli, and two other acid fast organisms, when incubated over alkalis in closed spaces, cannot be explained as due to dehydration of the medium, since growth occurs over more efficient dehydrating agents which are not carbon dioxide absorbents, such as sulphuric acid, calcium chloride, and glycerine. The only explanation of this phenomenon is that carbon dioxide in some way is a vital factor in growth.

Rhea, L. J.: Stand for Staining Blood Smears. Internat. Assn. Med. Mus., 1924, p. 91.

The author describes a stand for staining blood smears which is very simply prepared with the equipment in any laboratory and has the advantage of preventing evaporation of the alcohol during the staining with Wright's stain.

A wide-mouthed bottle is fitted with a cork, in which a hole is cut, just large enough to fit snugly into it the glass knob of the cover of a glass jar. In the base of this inverted cover two narrow platforms of glass are cemented at a convenient distance apart with reference to the ordinary glass slide, and slightly narrower than such a slide. The slide with the smeared preparation of blood to be stained is placed on these platforms, flooded with the Wright's stain in the usual manner, and the whole covered with a pane of glass. It is advisable to have a little methyl alcohol lying free in the base of the inverted cover so that during the staining process an atmosphere saturated with the alcohol is present and no evaporation occurs from the staining fluid.

White, C. P.: A New Method of Decalcification. Jour. Path. and Bact., London, 1923, xxvi, 425; Abstr. Bull XI: Internat. Assn. Med. Mus., May 4, 1925.

A saturated watery solution of citric acid is diluted 1:10 with water. A small quantity of methyl red and naphtholphthalein are added and then strong ammonia until the fluid is a clear yellow color. Too much ammonia turns the fluid green. The fluid contains about 6 per cent citric acid and should have a P_H of 6 to 8. Chloroform is added to prevent the growth of moulds. After decalcification, wash well in water before placing in alcohol.

The advantages are the use of a neutral solution for decalcification. The staining is unaffected and it seems to exert no harmful effect on the tissue.

Smith, J. L., and Rettie, T.: An Aldehyde Mordant for Fats and Lipoids. Jour. Path. and Bact., 1924, xxvii, 115; Abstr. Bull. XI: Internat. Assn. Med. Mus., May 4, 1925.

Preparation of the aldehyde solution:

In a flat-bottomed 400 c.c. flask put 25 c.c. of paraldehyde. To this add 25 c.c. of dilute HCl (equal parts water and pure HCl). Heat in an oven at 37° C. with frequent shakings until the paraldehyde is dissolved. This takes fifteen hours or longer and it is not safe to leave in the incubator overnight. The resulting pale brown solution is diluted with water, neutralized with NaOH and made up to 1000 c.c. (2.5 per cent paraldehyde). Adjust reaction to P_H 6 with acetic acid. Stored in dark bottles it keeps for months and reaction should be readjusted if changed, to P_H 6 with caustic soda. The tissues to be stained are fixed in 10 per cent formalin for twenty-four to forty-eight hours and not longer. Frozen sections are cut and mordanted in this solution at 37° C. for twenty-four to forty-eight hours. Wash in water and stain six to eighteen hours in 1 per cent hematoxylin in 0.5 per cent acetic acid. Differentiate in solution containing 1 per cent borax and 0.5 per cent potassium ferricyanide. The fat and lipoid globules alone remain dark blue.

Hirschfeld, H.: Experience with Oxidase and Peroxidase Reactions. Med. Klin., Berlin, 1924, xx, 249; Abstr. Bull. XI: Internat. Assn., Med. Mus., May 4, 1925.

The most convenient method of applying the oxidase reaction is that of W. H. Schultze.

Fixation of blood preparation in absolute alcohol and then treatment for about five minutes in a filtered mixture of equal parts of a 1 per cent aqueous solution of basic dimethylparaphenylenediamine (Merck or Schuchardt) and a 1 per cent strongly alkaline or alcoholic solution of alpha-naphthol.

The older the solution the shorter will be the time necessary for fixation. Finally, however, the dimethylparaphenylenediamine especially loses its action. If one wishes to preserve the preparations thus treated and counterstained with safranine fuchsin, or pyronin, they must be embedded in water glass, but even then their durability is limited.

For the peroxidase reaction Graham's modification has been found useful.

Fixation for about half a minute in a mixture of 1 part formaldehyde and 9 parts 95 per cent alcohol. Carefully wash off and then place for five minutes in a solution of a few grains of benzidine in 40 per cent alcohol to which 10 c.c. of 0.02 H_2O_2 have been added.

Staining follows in Loeffler's methylene blue or better, a highly concentrated Giemsa solution (ten to fifteen minutes' staining in a solution of 0.6 Giemsa in 10 c.c. Aqua destil).

This produces many beautiful preparations in which all normal and pathological leucocyte forms are easily differentiated.

Kinney, E. W., and Campbell, H.: Jahnel's Method for Staining Spirochetes in Nerve Tissue. Bull. XI: Internat. Assn. Med. Mus., May 4, 1925, p. 121.

The method following has given excellent results in tissues preserved for some time in formalin, but is not so satisfactory for freshly fixed tissue. The best results were obtained in tissues preserved for years (ten).

1. Wash pieces of formaldehyde or alcohol-fixed tissue from 2 to 4 mm. thick in distilled water for one to three days.

2. Pure pyridine one to three days.

3. Wash for two to three days in many changes of water until the pyridine odor almost disappears; this is important.

4. Allow the pieces to remain a "few days" (einige tage) in a 5 to 10 per cent formaldehyde solution, U.S.P.

5. Place in water again. (The time in water is not stated here; probably the washing should be thorough.)

6. Place in a 1 per cent solution of uranium nitrate (Merck) in distilled water for one-half to one hour in the incubator at 37° C. Lead-free glass wool may be used under the tissue to aid penetration but is not absolutely necessary. The uranium nitrate inhibits the staining of other elements of the nervous tissue.

7. Wash in distilled water for one day.

8. Ninety-six per cent alcohol three to eight days.

9. Distilled water until the block sinks.

10. Freshly prepared 1.5 per cent silver nitrate solution in an amber flask from five to eight days in the oven at 37° C.

11. Decant the silver nitrate solution; wash the tissue slightly in water and transfer to the following solution for one to two days.

4 per cent aqueous solution of pyrogallol	95 c.c.
Formaldehyde solution, U.S.P.	5 c.c.

"We have found it unnecessary to leave the tissue in any solution longer than the minimum time given. In Step 4 we leave blocks twenty-four hours in the formaldehyde solution. In Step 5 we wash twenty-four hours in frequently changed distilled water."

Of the brains examined which had an anatomic and histologic diagnosis of general paresis 58.3 per cent showed spirochetes by this method.

Boissevain, C. H.: A Method for Obtaining Single Colonies of Tubercle Bacilli. Am. Rev. Tub., January, 1926, xiii, 90.

"For the study of variation of virulence, or any other biological property of bacteria, it is important to use cultures derived from a single organism. Ordinary cultures, made by planting millions of bacilli and securing a massive confluent growth, are likely to contain a mixture of strains which may differ widely from one another. With organisms which grow vigorously on artificial media it is easy, by simple planting, to get colonies, which we are reasonably sure have grown from one individual; or we may use the Barber Technique (1) and be quite certain; but with the tubercle bacillus such methods usually give no growth. In the hope of finding a satisfactory method the following procedures have been tried, with encouraging results:

"A: A rabbit is bled from the carotid artery and the blood received into paraffined centrifuge tubes packed in ice, and centrifuged at once. A rather light suspension of tubercle bacilli in 1.5 per cent sodium-chloride solution is prepared, filtered through sterile filter paper, and centrifugated for a few minutes. One drop of the supernatant suspension is mixed with 1 cc. of the rabbit plasma in a sterile tube, stoppered with paraffined cotton, slanted to form a thin layer, and left to coagulate in the incubator. Coagulation occurs in about ten minutes, and after that the tubes should be left lying on their sides. In an upright position all the serum is pressed out of the coagulum and collects at the bottom of the tube. In about two weeks many small discrete colonies can be seen *in* the plasma, but not on the surface. These can be transplanted or used at once for inoculation. This confirms an observation of A. E. Wright on the multiplication of tubercle bacilli in capillary tubes filled with plasma.

"B: Rabbit serum, with the addition of an equal volume of 3 per cent agar in distilled water at 50° C., but without peptone, salt, or glycerine, is substituted for the plasma. With this method, the results have varied. Serum from some rabbits has yielded a few colonies; serum from others none; in no instance has the growth been as abundant as with plasma.

"C: Freshly ground rabbit liver is added to the rabbit serum of method *B*, in the proportion of about 1 to 20.

"In this medium the tubercle bacilli grow as well as in plasma. The liver of a tuberculous guinea pig may be used, and primary growth be secured in the form of single colonies. There would, however, be more doubt of the colonies having developed from a single bacillus than when a light filtered and centrifugated bacillary suspension is used.

"D: In 'hormone agar' plus rabbit serum, in equal parts, colonies develop abundantly. If the serum is omitted, a few colonies sometimes appear. Experiments are now in progress to ascertain what substance present in tissue and in plasma is so favorable to the growth of the tubercle bacillus."

Hackenthal, H.: A Modified Schuffner's Blood Stain. Deutsch. Arch. f. klin. Med., Leipzig, 1924, cxiii, 276.

The author gives a staining method designed to avoid the drawbacks of the usual smear preparation. Destroyed or artificially distorted cells are unusually few and cells and nuclei maintain their living form. All the cells which are differentiable in the smear preparation are also easily distinguishable.

Technic.

Schuffner's Solution

NaCl	4.0
Borax	0.1
Concentrated carbolic acid	3.0
Formalin	1.0
Aqua destil	1000.0

To every 2 c.c. of Schuffner's solution, 1 to 2 drops of saturated aqueous methylene blue solution (methylene blue medicinal Hochst) and 1 drop of saturated aqueous dahlia solution (G. A. Hesterberg, Berlin, Lindenstrasse 39). One-tenth cubic centimeters of this solution, which must be freshly made up, is placed in a small cylindrical glass vessel made from a 4 or 5 cm. long piece of glass tubing of 0.5 to 0.7 cm. bore. To this add 20 c.mm. of blood, using a capillary pipette from a Sahli's or Gower's hemoglobinometer, which must first be washed out with Schuffner's solution or the greater number of the leucocytes will stick to its walls. A glass capillary tube first washed through with Schuffner's solution is used to mix the blood and stain thoroughly. A small drop of the mixture is placed on a clear slide and covered by a cover-glass. The drop must be of such a size that it will reach to the edge of the cover-glass and yet the cover-glass must not float on it. The edge of the cover-glass is then "ringed" with vaseline to hinder evaporation and the resulting injury to the blood cells. The blood cells will now lie almost in an optical plane

without streaming movements. In warm weather staining of the leucocytes proceeds more quickly than in cold weather.

Erythrocytes remain unstained except those which in smear preparation appear darkly colored as in metachromasia; these become light blue to light violet. Nuclear fragments of erythroblasts, stippling inclusive of Schuffner's stippling in malaria are well stained. Poikilocytosis and anisocytosis are recognizable without exception.

Hall, M. W., and Lacy, G. R.: The Mechanism of the Russell Double Sugar Tube. Jour. Infect. Dis., January, 1926, xxxviii, 14.

While the acids produced in the fermentation of dextrose in the Russell double sugar medium are mainly, if not entirely, volatile, their diffusion out of the medium is not the cause of the alkaline reaction shown on the slant when typhoid and related organisms are grown. This reaction is due to excess of alkaline substance produced from nitrogenous elements in the presence of oxygen.

In the absence of oxygen a "mother substance" is formed which rapidly becomes alkaline when exposed to oxygen.

The ultimate reversion in the butts of Russell tubes is the result of the diffusion into the butts of oxygen rather than of the alkali formed in the slant portion of the tube.

The paratyphoid and colon groups appear capable of producing an alkaline substance under anerobic conditions, thus evidencing a distinct difference in metabolism which may prove worthy of further study. The chemical nature of the substance which is so easily oxidized into an alkaline substance is unknown.

Stevens, F. A., and Dochez, A. R.: Occurrence of Throat Infections with Streptococcus Scarlatinae without a Rash. Jour. Am. Med. Assn., April 10, 1926, lxxxvi, 1110.

A study of an epidemic of streptococci (hemolytic) sore throat during 1924-25 in a hospital demonstrated that:

1. Scarlatinal infection of the throat may occur without a rash.

2. This type of infection may occur in individuals showing negative skin reactions to scarlatinal toxin.

3. The Dick test is not a reliable index of immunity to such throat infections with streptococcus scarlatinae.

4. In the series of cases observed agglutination reactions with scarlatinal serum and toxin production are closely parallel.

5. There is no antigenic relationship between strains of hemolytic streptococci from acute streptococcus pharyngitis.

Hirsch, E. F.: Separation of a Soluble Specific Substance from Hemolytic Streptococci. Jour. Infect. Dis., December, 1925, xxvii, No. 6, 523.

The method used was as follows:

Broth was prepared with fresh placenta tissue, 1 pound of the minced tissue per liter of water. To the filtered portion, 1 per cent peptone and 0.8 per cent sodium chloride were added. After heating to boiling, the reaction was adjusted to P_H 7.6, the mixture filtered and sterilized. To each 100 c.c. by volume, approximately 20 c.c. of sterile ascitic fluid was added. Containers of this medium were inoculated with a strain of Streptococcus hemolyticus, incubated at 37° C. for 48 hours, and the growth of bacteria recovered by centrifugation. The sediment so obtained was washed quickly in 0.9 per cent salt solution. To the bacteria N/10 NaOH was added, (enough to make a turbid suspension), and the mixture transferred to a 250 c.c. separatory funnel. Small quantities of ether added to this were thoroughly shaken into the mixture, and presently formed with the bacterial suspension a fairly stable emulsion. The emulsion so started was built up by adding small quantities of ether and shaking. When the amount of ether added exceeded that capable of remaining emulsified, the excess was removed, and the separatory funnel with its content stood upright for about 1 hour. At the end of this time two layers

formed in the funnel—a lower, clear layer, and an upper, gummy layer. The clear layer was removed, and the gummy layer above, containing the emulsified ether, was broken up by rotating the separatory funnel. The ether, separating by this manipulation, was poured off or evaporated with a current of air. This left a turbid liquid which was filtered, the filtrate centrifugalized and the clear or slightly opalescent liquid electrodialyzed against distilled water. When the reaction of the liquid came to the proper P_H by electrodialysis and adjustment with dilute acid or alkali there was a turbidity and, on standing, a copious flocculation of white particles. A reaction slightly acid or alkaline stabilized the particles, and there was no flocculation. However, the acid solution was more opalescent than the alkaline. The white flocculent precipitate was recovered by centrifugation, washed repeatedly in distilled water, used as such, or dried in a vacuum desiccator. Washing with distilled water usually results in a loss of some material, because the P_H of the distilled water used in washing does not coincide with the P_H (isoelectric) of flocculation. Finally the washed material may be reduced to a brown white powder.

The material so obtained is soluble readily in alkaline solutions, and in this respect corresponds with extracts obtained from other bacteria. It is also soluble to a less extent in dilute acids (N/100). Increasing the acidity renders the solution more opalescent, and heating seems to change the solubility in acid solutions. The moist precipitate is slightly soluble in 0.9 per cent sodium chloride solution, it is insoluble in 95 per cent alcohol, acetone and ether. A solution of the precipitate in water containing a few drops of N/100 sodium hydroxide reacts with Millon's protein test, but not with the biuret test. There is no reduction of Haines' solution, no precipitation with 95 per cent alcohol, or acetone. On heating alone and with dilute acetic acid there is a slight haze. One preparation not regarded as entirely pure contained about 10 per cent nitrogen. A solution gave precipitin reactions with an antistreptococcus rabbit serum in dilutions as high as 8 parts in 100,000. This antistreptococcus immune serum has only a moderate titer. Solutions of many preparations made in that way gave precipitin reactions with antistreptococcus serums and with the serum of a rabbit receiving repeated injections with solutions of other similar preparations. There is no hemolysis when isotonic solutions of these preparations are mixed with red blood corpuscles.

DeAua, E. A. S.: Gonococcus Vaccines with Urotropin. Rev. Med., Barcelona, October, 1925, iv, 374.

The author reports his experience with vaccines preserved with urotropin which apparently delays the aging of vaccines and preserves them.

It was found that vaccines with urotropin gave, in small doses, a much stronger reaction than plain vaccines. The small dose of urotropin incorporated in the vaccine acts solely by preserving the initial strength of the vaccine; in other words, such vaccine keeps fresh for a much longer period.

The proportion of urotropin in the vaccine is not stated.

Garrod, L. P.: On Sulphemoglobinemia. Brit. Quart. Jour. Med., October, 1925, xix, 86.

In two cases of sulphemoglobinemia the feces gave a growth of the nitrosobacillus and treatment with a vaccine of this organism produced prompt results.

The method used for isolation of the organism was as follows:

A weighed amount of feces is completely emulsified in sterile distilled water and a series of dilutions made from each of which 0.1 c.c. was plated on litmus-lactose-agar plates. The plates should be spread for at least 30 seconds to permit complete absorption of the fluid by the medium.

Three such series of plates are made, there being six in each series representing amounts of feces from 1/50,000 to 1/10,000,000 gram. The plates are then incubated at 37° C., 30° C., and 20° C. In those incubated at 37° C. only colon bacilli and streptococci were obtained; in the others the coliform colonies are smaller and the nitrosobacillus colonies appeared on the fourth day.

The organism was an oval, gram-negative, nonmotile bacillus, growing slowly on all media, fermenting no sugars, reducing neutral red, liquefying gelatin, and forming nitrites.

The author urges a search for this organism in similar cases.

Falk, I. S., and Yong, S. Y.: The Influence of Certain Electrolytes and Nonelectrolytes on the Bile Solubility of Pneumococci. Jour. Infect. Dis., January, 1926, xxxviii, 1.

Washed suspensions of pneumococci in distilled water are usually bile soluble.

Chlorides with monovalent cations (Na, K, NH_4, Li) in relatively low concentrations inhibit bile solution of washed pneumococci.

In higher concentrations these chlorides do not inhibit and may accelerate solution.

Chlorides with divalent cations (Ca, Ba) behave differently and inhibit bile solution of pneumococci more effectively in high than in low concentrations.

Of the anion series tested NaOH and Na_3PO_4 are cytolytic for pneumococci; Na_2HPO_4, NaH_2PO_4, Na_2SO_4, and $NaNO_3$ are not cytolytic.

Cytolysis by NaOH and Na_3PO_4 appears to be a function of pH concentration.

Peptone, gelatine, and ovalbumin appear to inhibit cytolysis by bile in the same manner as $CaCl_2$ and $BaCl_2$. The inhibitory action increases with concentration.

Kerr, D., and Mason, V. H.: The Hemochromogen Crystal Test for Blood. Brit. Med. Jour., Jan. 23, 1926, p. 134.

The advantages of Takayama's method for the detection of blood by the formation of hemochromogen crystals are extolled.

The test solution consists of: 10 per cent sodium hydroxide solution, 3 c.c.; pyridin, 3 c.c.; saturated solution of grape sugar, 3 c.c. and distilled water, 7 c.c.

The sodium hydroxide acts as a blood solvent and the grape sugar as a reducing agent. The solution keeps for about two months.

On the addition of two or three drops of this solution to a small piece of the suspected material on a slide in the cold and covering with a cover-glass, salmon-pink crystals appear within two or three minutes which can be clearly seen under the low power (200 to 300 magnifications).

At the same time the color changes through green-brown, dark red, to pink thus indicating the formation of hemochromogen and confirming the test.

The crystals usually appear within one to six minutes but a negative test should be observed for 30 minutes.

If the slide be heated just to bubbling the crystals appear almost at once.

There is no danger of overheating.

The crystals are single, shallow, rhomboids of salmon-pink color. When on their side they appear like dark, single lines.

They are often very large.

The crystals may be obtained from material which has been heated, washed or contaminated with rust.

Milroy, G. A.: Method for Estimation of Glucose in the Blood. Jour. Biochem., 1925, xix, 746.

1. Precipitate the proteins from 1 c.c. of blood by the Folin-Wu method. The blood is, hence, diluted 1:10.

2. After standing 30 minutes, filter and place 5 c.c. of filtrate in a 15 c.c. graduated tube.

3. Into five graduated 15 c.c. tubes of 12 mm. bore introduce the following amounts of 0.02 per cent glucose solution: 1.5, 1.8, 2.1, 2.5 and 3 c.c.

4. Add to each tube 1 c.c. of 0.4 per cent aqueous 1.5 nitroanthraquinonesulphonic acid and 2 c.c. of potassium carbonate solution (50 mg. per 100 c.c.).

5. Dilute all tubes to 10 c.c. mix, immerse in boiling water for 8 minutes, cool and make the volume 12.5 c.c.

6. Compare the red tint of the blood filtrate with the glucose solutions.

The minimal error in a sample of blood containing 0.1 per cent glucose is 4 per cent.

Belding, D. L.: Notes on the Etiology and Epidemiology of Impetigo Contagiosa Neonatorum. Am. Jour. Obst. and Gynec., January, 1926, xi, 1.

The study corroborates the etiologic relation of a strain of Staphylococcus aureus to impetigo contagiosa neonatorum. The strain failed to produce skin lesions in guinea pigs or rabbits but caused a nonvesicular inflammatory reaction in the skin of an adult and a typical exfoliating lesion in the skin of the infant from whom it was isolated. The cultural reactions, except for a questionable minor difference in the rate of carbohydrate fermentation, are the same as those of the ordinary staphylococcus.

The variation in epidemics is probably due to variations in the virulence of the infecting strain; variations in the clinical symptomatology of infants and adults are probably due to the resistance of the host.

The primary prophylactic measure in a hospital epidemic is the individual handling of the well infants, as early cases are capable of transmitting the infection before a diagnosis is made.

Joekes, Th.: Cultivation of the Spirillum of Rat-Bite Fever. The Lancet, London, Dec. 12, 1925.

Successful results were obtained with a medium consisting of Loeffler's blood serum on top of which was poured 10 c.c. of Vervoort's medium (peptone 1 gram; normal phosphoric acid 3 c.c.; distilled water 900 c.c.).

The most favorable reaction is P_H 7.2 to 7.6. The reaction of Vervoort's medium is originally P_H 6.6 but, after warming for 15 minutes in contact with the serum slope at 37° C. alters to P_H 7.0 to 7.2.

Sanders, G. H.: Isolation of Monilia from the Skin Scales, Mouth, and Sputum in Psoriasis. The Lancet, London, Dec. 12, 1925.

An organism belonging to the monilia was isolated in conjunction with streptococci from the areas noted above.

Tunnicliff, R., and Hayne, A. L.: Further Studies on a Diplococcus from Measles: Prevention of Measles by Immune Goat Serum. Jour. Infect. Dis., January, 1926, xxxviii, i, 48.

Report of further studies on a filter-passing, gram-positive, green producing diplococcus isolated from the blood in the early eruptive stage of measles by Tunnicliff in 1917.

The present paper reports the successful prevention of measles in rabbits by the injection of immune goat serum.

Convalescent goat serum also protected human beings against measles as effectively as human convalescent serum when injected on the first and second days after exposure.

Holman, W. L.: An Error in Acid Fast and Gram Staining Due to Petrolatum. Arch. Path. and Lab. Med., March, 1926, i, No. 3, p. 390.

Smears made from lesions in which petrolatum has been used must be treated with xylol or some other solvent before staining. If this precaution is omitted false gram-positive or acid-fast reactions will be obtained.

The possibility of this error must be recognized when petrolatum has been used on catheters or when smears are made from anaerobic tubes in which petrolatum is used on the surface of the medium.

Ramos, Passos J. R.: On Lowenstein's Procedure for Direct Isolation of the Tubercle Bacillus from Sputum. Compt. rend. Soc. Biol., Paris, Dec. 18, 1925, xciii, 1552.

Technic.—The sputum is placed in a centrifuge tube and five times its volume of a 15 per cent solution of sulphuric acid is added. The tube is allowed to stand for 20 minutes, shaking occasionally until a homogenous mixture is obtained. At the end of this time the sputum is centrifuged at high speed for 15 minutes. The liquid is removed and the sediment is washed with sterile physiologic salt solution. The centrifugation and washing are repeated two more times. The liquid in the tube is then cultured on glycerinized potato, five tubes of the medium being used for each specimen. The tubes are well stoppered and paraffined and are then placed in the incubator at 37° C.

The method proved successful in the hands of the author in 104 microscopically positive and in two out of 33 microscopically negative specimens.

Tunnicliff, R.: Further Studies on a Diplococcus in Measles: A Measles Skin Reaction. Jour. Infect. Dis., September, 1925, xxxvii, No. 3, p. 193.

Anerobic dextrose broth cultures of a diplococcus found by the author in measles, killed by 0.5 per cent phenol, appear to produce a skin reaction in persons who have not had measles, but not in measles patients after the appearance of the eruption, or in 96 per cent of persons who give a history of measles.

The measles antigen is neutralized in persons who have not had measles by convalescent human measles serum, but not by the serum of a person with a negative history of measles.

Rabbits immunized against measles fail to react.

Greenough, R. B.: Varying Degrees of Malignancy in Cancer of the Breast. Jour. Cancer Research, December, 1925, ix, No. 4, 453.

A report of a study of cases of cancer of the breast in the Massachusetts General Hospital during 1918, 1919 and 1920 in accordance with the principle of McCarty and Broders that the degree of malignancy may be estimated by loss of differentiation and increase of productive characteristics, dividing the tumors into three groups of high, medium, and low malignancy.

The results are thus summarized:

1. The degree of malignancy of a given case of cancer of the breast can be determined with reasonable accuracy by study of the histology.

2. A classification of high, medium, and low degrees of malignancy can thus be made which is of prognostic value and of aid in the evaluation of therapeutic measures.

3. In estimating the degree of malignancy the following factors are of importance:

(a) Degree of differentiation, as shown by arrangement of cells around an open gland, (adenocarcinoma).

(b) Degree of secretory activity of cell protoplasm as shown by vacuoles and droplets of mucoid material.

(c) Uniformity of size of cells and nuclei as opposed to variation in size.

(d) Absence or presence of hyperchromatic changes in nucleus and few or many mitotic figures, and whether irregular or not.

(e) High malignancy is shown by cells and nuclei of irregular shape and size without secretory function, and arranged in solid columns, large or small, together with numerous and irregular mitoses and hyperchromatism. The extreme degree of these changes is pleomorphism.

(f) A tumor of adenomatous arrangement with uniform sized cells and nuclei, few mitoses, and absence of hyperchromatism, indicates low malignancy.

4. A high degree of round cell infiltration appears to indicate a considerable degree of cell degeneration and is not to be relied upon as an indication of the resistance of the individual to the cancer growth.

5. Hyalinization of the stroma does not indicate active resistance to the tumor growth but is rather a factor of the age or previous condition of the mammary tissue in which the tumor lies.

The paper is illustrated with sixteen microphotographs showing the appearances described.

Cruikshank, R.: B. Bifidus: Its Character and Isolation from the Intestine of Infants. Jour. Hygiene, December, 1925, xxiv, 241.

The following method was used for the isolation of B. bifidus from the feces:

A deep tube containing 20 c.c. of 1 per cent lactose or glucose broth neutral to litmus, and containing a small piece of fresh, sterile rabbit kidney, and sealed with vaseline, is inoculated by means of a capillary pipette with 0.5 c.c. of a fairly opaque fecal emulsion. The vaseline is melted to seal the track of the pipette and the tube incubated 6 to 8 days at 37° C.

During the first few days the gas formed is conveniently expelled by melting the vaseline seal.

Plates of 1 per cent glucose-agar or Loeffler's serum are streaked and incubated aerobically and anaerobically.

After 48 hours at 37° C. small, glistening grayish colonies of pin-head size are seen, generally on the anerobic plate, occasionally aerobically.

The only other organism likely to be found is the enterococcus which forms larger, whitish colonies readily distinguishable from the diphtheroid B. bifidus colonies.

Subcultures are made aerobically in glucose-agar slants, glucose broth, and milk. Rich inoculation is advisable.

Harris, J. S.: A Simple Test of Diagnostic Value in General Paresis. Brit. Med. Jour., Jan. 23, 1926, p. 136.

Harris reports his results with the acetic anhydride-sulphuric acid test described by Grossman in 1925 (Jour. Mental Sc., xxi, 439).

The method follows:

To 1 c.c. of spinal fluid in a small test tube add 0.3 c.c. of acetic anhydride. Shake well and add carefully, drop by drop, 0.8 c.c. of concentrated sulphuric acid. The tube is then held against a white background. A lilac tint indicates a positive reaction; a brown-yellow or red-yellow constitutes a negative reaction. The lilac color of a positive reaction may appear and disappear within a minute or so, hence the reading should be made at once.

The test is presumed to be due to the presence of cholesterol in the fluid.

In 180 cases of various mental diseases Harris found this reaction positive in 97 per cent of paretics and negative in all the control conditions.

Baer, J. L., and Reis, R. A.: The Sedimentation Test in Obstetrics and Gynecology. Surg., Gynec., and Obst., May, 1925, p. 691.

Using the technic described by Linzenmeir the authors report a study of the sedimentation test in 100 cases of pregnancy and in various gynecologic conditions.

They conclude:

1. The sedimentation test is of no value in the diagnosis of pregnancy.

2. With pelvic pathology a sedimentation time over 2 hours conclusively rules out pelvic infection.

3. The rate of sedimentation is directly proportional to the virulence of the infection.

4. The test is of value in determining the safe time for operation and seems a more delicate prognostic index, good or bad, than either the leucocyte or temperature curve.

(A further communication by the same authors—The Sedimentation Test in Gynecology, Am. Jour. Obst. and Gynec., September, 1925, x, 3—was abstracted in the January, 1926, issue of this Journal.)

Gray, S. H., and Feemster, L. C.: Compensatory Hypertrophy and Hyperplasia of the Islands of Langerhans in the Pancreas of a Child Born of a Diabetic Mother. Arch. Path. and Lab. Med., March, 1926, i, No. 3, 348.

A case is reported in which the pancreas of a child born of a diabetic mother contained approximately twenty-four times as much insular tissue as the normal pancreas.

The increase in maternal blood sugar is suggested as the stimulus which called forth the increase in insular tissue in the pancreas of the child. Hypoglycemia, the result of this insular hypertrophy and hyperplasia, may play a rôle in the sudden death of the baby.

Associated with the hypertrophy of the islands of Langerhans was an hypertrophy of many medullary cells of the suprarenals.

Smith, L. W.: Four Unusual Malignant Tumors of the Liver. Arch. Path. and Lab. Med., March, 1926, i, No. 3, 365.

In a paper illustrated by one colored plate and eleven microphotographs are described cases of (a) primary carcinoma; (b) metastatic leiomyosarcoma; (c) melanotic sarcoma; and (d) angiosarcoma.

Studies on the Bacteriophage of D'Herelle.

The lack of adequate direct evidence as to the nature of the active principle causing transmissible lysis is responsible for the existence of numerous hypotheses attempting to explain the mechanism of the bacteriophage phenomenon.

Olsen and Yasaki (Ztschr. Hyg. u. Infect., 1924, cii, 540) claim to have demonstrated that the bacteriophage is a volatile chemical substance; Bronfenbrenner and Karb (Is the Lytic Principle Volatile? Jour. Exper. Med., January 1925, xli, No. 1, 73) have failed to confirm these results, attribute them to the transfer of minute droplets from the filtrate to the distillate, and conclude that the lytic principle is not volatile.

The same authors (Effect of Alcohol on the Bacteriophage of D'Herelle, Jour. Exper. Med., September, 1925, xii, No. 3, 419), having observed that, in many instances precipitates by alcohol from lytic filtrates manifested appreciable lytic action, investigated this question and found:

1. When bacteriophage is precipitated by alcohol at room temperature its activity rapidly and progressively decreases until it is totally destroyed in 6 to 24 hours.

2. If precipitation is carried out at 7° C. the destruction is considerably slower.

3. The inactivation of bacteriophage by alcohol seems analgous to the alcoholic inactivation of certain enzymes and toxins.

In a study of the quantitative evaluation of the activity of bacteriophage Bronfenbrenner and Karb (Some of the Factors Determining the Number and Size of Plaeques of Bacterial Lysis on Agar, Jour. Exper. Med., October, 1925, xlii, 4, 483) confirm the fact that the lytic principle is distributed in active solution in a state of indivisible which permits its quantitative evaluation by serial dilution as well as by plating on agar, the latter method, however, often giving lower readings. This discrepancy is due to adsorption of the lytic principle on agar. When the agar concentration is increased from 0.3 to 2.5 per cent, the number of plaeques is reduced more than 100 times. The size and number of plaeques is also influenced by the condition of the culture employed in titration, the number of susceptible bacteria, etc. Under carefully controlled conditions the size of the plaeques is found to be determined by the character of the lytic filtrate; thus, with lytic agents acting on more than one species of bacteria, the size of the plaeques remains constant regardless of the bacterial substratum used for the production of the active filtrate.

D'Herelle maintains that there is but one bacteriophage acting on all bacterial species.

Bronfenbrenner and Karb, (Concerning the Oneness of the Bacteriophage, Jour. Exper. Med. December, 1925, xliii, 6, 821), found different lytic filtrates differently affected by changes in hydrogen-ion concentration, that these differences persisted during

repeated passages of lytic filtrates through cultures of bacteria other than those against which they were primarily active, and consider this evidence in favor of a multiplicity of bacteriophages.

The addition of neutral salts to lytic filtrates results in an increased rate of inactivation when alcohol is added, (Bronfenbrenner, J. J., and Karb, C.: Effect of Electrolytes on the Rate of Inactivation of Bacteriophage by Alcohol, Jour. Exper. Med., January, 1926, xliii, No. 1, p. 71), the salt effect being more marked the higher the valency of the cation.

Conversely, the removal by dialysis of salts originally present, renders the lytic agent less sensitive to alcohol.

It appears that the action of alcohol is dependent upon the precipitation of coagulable constituents and not upon a direct toxic action on the bacteriophage which is evidence in favor of its chemical nature.

Pergola, M.: Culture Medium for B. Diphtheriae. Ann. d'igeine, June-July, 1925, xxxv, 537.

Pergola describes the following as an enrichment substance to be added to agar for the isolation and culture of the diphtheriae bacillus:

Yolk of egg	75 gm.
Glycerine	12.5 gm.
8% NaCl solution	12.5 gm.
Potassium tellurite	0.1 gm.

In this solution the egg yolk will be preserved and sterile. To 100 c.c. of agar add 20 c.c. of this solution.

BOOK REVIEWS

(Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.)

*Preventive Medicine**

A BRIEF and eminently practical presentation of the salient features of modern preventive medicine.

The author believes—and there is much in justification of his contention—that physicians at large are neglecting their opportunities in the field of preventive medicine and public health. In part this may be due to lack of succint and accessible information in this field.

In the present volume is presented the minimum knowledge of the subject which a student or practitioner of medicine should be expecetd to possess.

The first 264 pages are devoted to a very clear, though compact, discussion of epidemiology.

This is followed by a discussion of deficiency diseases (21 pages), occupational diseases (11 pages), a chapter on diseases arising from the puerperal state, and one on diseases transmitted from parent to offspring.

*Preventive Medicine. By Mark F. Boyd, M.D., C.P.H., Member Field Staff, International Health Board of Rockefeller Foundation; formerly Professor of Bacteriology and Preventive Medicine in the Medical Department of the University of Texas. Second Edition. Revised. Pp. 429. 135 illustrations. Cloth. Price $4.00 net. W. B. Saunders Co., Philadelphia and London, 1925.

Separate sections are devoted to special aspects of hygiene and sanitation (33 pages), demography (25 pages), and a final section on public health administration (11 pages).

The volume amply fulfils its purpose and should be in the hands of every practitioner.

*The International Association of Medical Museums**

THIS eleventh bulletin of The International Association of Medical Museums within a limited space comprises a storehouse of information of interest alike to pathologists, curators of museums, and technicians.

In addition to a report of the various activities of the association, there are twenty original articles, covering a variety of subjects from the histologic findings of a tumor preserved in alcohol for 118 years to new or improved technical procedures.

There is also a very extensive and useful department of technical abstracts.

The publication very ably reports the activities in its special field.

Memoranda of Toxicology†

BASED upon Tanner's *Memoranda of Poisons*, this little handbook serves a distinct purpose.

After a general consideration of the mode of action and classification of poisons, including some very clearly written chapters on the diagnosis of poisoning, the duties of the practitioner in cases of poisoning, and the treatment and detection of poisons in general, various irritants and poisons are then considered in detail.

The diagnosis and treatment of poisoning is a subject of some difficulty and while there are many books available on this subject not all are sufficiently compact or concise to furnish readily available information.

The present volume in a small space presents a large amount of eminently practical information.

The newer sources of poisoning, such as tetraethyl lead, asphyxiating gases, barium salts (as used in x-ray work) are included and all the newer data concerned with diagnosis and treatment has received consideration.

Each poison is briefly but satisfactorily discussed under the following headings: Nature of Action, Symptoms, Postmortem Appearances, Treatment, and Tests. While it is beyond the province of the book to discuss in detail the chemical detection and determination of poisons, under "tests" are given ready and simple methods for their general recognition.

Addenda, contributed by Dr. Leffman, include discussions on Bites and

*Bulletin No. XI of The International Association of Medical Museums and Journal of Technical Methods. Pp. 151. Illustrated. Paper. Price $3.00. Paul B. Hoeber, Inc., New York.

†Memoranda of Toxicology. By Max Trumper, A.M., formerly Lecturer on Toxicology, Jefferson Medical College, and Henry Leffman, M.D. Emeritus Pathologic Chemist Jefferson Medical College. Flexible pocket size. Pp. 230 Price $1.50. P. Blakiston's Son and Co., Philadelphia.

Stings, Drug Addiction, The Poison Rum Problem, First Aid, What Is a Poison, and Tobacco as a Medium for the Administration of Patent Drugs.

For the medical student and industrial or general practitioner this little book will be most useful.

*Aviation Medicine**

THE specialty of aviation medicine represents almost entirely a development of the last twenty years. The present volume is the first textbook written on this subject in America, and the first one written anywhere since the late war. Great progress is constantly being made along the lines of aviation, and its probable future importance in civil as well as in military life renders this volume of peculiar interest as a kind of practical pioneer in a subject which has enthralled the human mind from the time of its earliest conception of birds or angels or dragons or other similar creatures which could soar in the air, often to the disadvantage of man, and always with the excitement of his envy.

The author has attempted to consider all phases of the subject, to correlate the results of various workers and to present a handbook for the use of those interested in the subject. It is intended that the book shall be sufficiently technical to be of use to the Flight Surgeon, but not too technical to interest the physician who has not worked in the subject.

The book is made up of three sections, the first of which deals with the selection of the flyer, the second with the physiology of aviation including the classification of the flyer, and the third with the care and maintenance of the flyer.

The book contains seventeen chapters, a supplement, and a splendid bibliography of some twenty-six pages. A summary conception of the wide range of the subject matter involved may be obtained from a brief consideration of the chapter headings which are as follows: Section one contains chapters on general physical qualifications; the eye in aviation; the nose, throat, and ear in aviation including a consideration of equilibrium; neuropsychic factors, and reaction time. Section two contains chapters on the effects of altitude on the respiratory and circulatory systems; the effects of altitude on the heart; psychologic effects of altitude; an altitude classification test; other tests for altitude, and effects of wind, cold, and speed. Section three contains chapters on fatigue, staleness, and physical fitness; protective devices; the flight surgeon; aviation accidents; airplane dope poisoning, and civilian flying, including the medical requirements of the international congress for air navigation. There are thirty-five illustrations. The book is well written, the language is terse and to the point, and a strong military atmosphere pervades almost every page. One is inclined to wonder whether or not this last feature bears any relation to the relatively small number of references to the German literature which the bibliography contains. As a whole the book is a valuable contribution and undoubtedly will be widely used by all medical men who are concerned with aviation either in military or in civil life. —*D. E. J.*

*Aviation Medicine. By Louis Hopewell Bauer, M.D., Major, M.C., U.S.A., Commandant of the School of Aviation Medicine. Pp. 241. Cloth. Price $7.50. Williams & Wilkins Co., Baltimore, 1926.

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EDITORIALS

Alkalosis

ALTHOUGH the term acidosis has been in common clinical use for many years, its antithesis, alkalosis, was first employed about ten years ago. Definite proof of the clinical existence of alkalosis has, however, only recently been presented.

Ever since the administration of sodium bicarbonate found common therapeutic use, cases have occasionally been noted where its administration has given rise to disturbing clinical symptoms. It is presumably for this reason that several prominent internists have long been strongly opposed to its use in combating the acidosis of diabetes. With the introduction of the Van Slyke method of estimating the blood bicarbonate, it has been recognized that high figures for the blood CO_2 might be encountered not only after alkali administration but also following obstruction high up in the alimentary tract. Since it was originally believed that acidosis conditions remained compensated until shortly before death, there was no reason to suppose that

conditions with high bicarbonate findings should be uncompensated, i.e., show a high P_H. With the introduction of satisfactory methods of estimating the hydrogen-ion concentration of the blood, however, it was observed that acidosis conditions became uncompensated much earlier than had been supposed. This quite naturally called attention to the opposite condition, uncompensated alkalosis.

It has required considerable time to correlate the various observations bearing on this acid-base balance. The studies of Hasselbalch, L. J. Henderson, Yandell Henderson, Van Slyke, and their coworkers, in particular, have done much to clarify our conception of this subject. Through their work it has come to be recognized that there is a definite relation among three interdependent variables, sodium bicarbonate, carbonic acid and P_H, the determination of any two permitting the calculation of the third. Stated in another way, the P_H is a function of the ratio $\frac{NaHCO_3}{H_2CO_3}$. Anything that will raise the sodium bicarbonate or lower the carbonic acid will raise the P_H, and, conversely, anything that will lower the sodium bicarbonate or raise the carbonic acid will lower the P_H. Several years ago Van Slyke[1] gave us a very clear presentation of this general problem.

Alkalosis may result from either an alkali excess or CO_2 deficit. If the organism is unable to compensate for this by keeping the ratio between the bicarbonate and carbonic acid constant at about 19 to 1, we have an abnormally high P_H and a condition of uncompensated alkalosis. The condition of high bicarbonate with normal P_H is referred to as compensated alkalosis. This may result not only from compensated alkali excess but also from CO_2 excess. As a matter of fact the latter condition was one of the first to have been clearly recognized. In 1920 Scott[2] showed that in emphysema the deficient ventilation of the blood in the lungs was followed by a compensatory retention of bicarbonate.

Conditions of uncompensated alkalosis are of the greatest interest and clinical significance, however, although where the alkalosis is due to an alkali excess it is almost invariably preceded by a condition of compensated alkalosis, i.e., high bicarbonate and normal P_H. Four types of uncompensated alkalosis have been definitely recognized clinically, namely, that following (1) sodium bicarbonate administration, (2) vomiting, particularly in "high-up" interstitial obstruction, (3) over ventilation due to fever, and (4) x-ray or radium therapy. The first two of these conditions are due to alkali excess, the third is due to CO_2 deficit, while in the fourth the uncompensation may occur with a perfectly normal blood bicarbonate.

Although alkalosis is much less common clinically than acidosis, it is very important to recognize since the condition is much more difficult to treat than acidosis. The symptoms are rather difficult to recognize, and in the past they have often been confused with acidosis, thus leading to therapy that directly aggravates the condition. One case came to my attention where three different men separately made a diagnosis of acidosis on the basis of the nausea and vomiting and each prescribed the same therapy, namely, gastric lavage and bicarbonate. The more prominent clinical symptoms may be given, as headache, lassitude, nausea, vomiting, fever, and in severe cases tetany.

Since vomiting and fever may lead to alkalosis, there exists a vicious circle; and it sometimes is difficult to recognize which is the cause and which the result. Ellis[3] has well described the clinical symptoms as follows: "The patients are unduly introspective and nervous. They are irritable and complain of trifles. There is headache, nausea and vomiting, dizziness, vertigo, and light-headedness. They may complain of aching pains in the muscles and joints. There is weakness followed by absolute prostration. They become apathetic, drowsy, and are aroused with difficulty, and finally tetany and convulsions may supervene."

Toxic manifestations following the administration of sodium bicarbonate have been noted by a number of investigators in recent years, although Binger, Hastings and Neill[4] were among the first to definitely establish the presence of an uncompensated alkalosis. In 1923 they reported observations on a case in which the P_H was raised to 7.55 (normal P_H 7.35 to 7.43) after bicarbonate administration. The following year Myers and Booher,[5] and Kast, Myers and Schmitz[6] presented data on 20 cases of uncompensated alkalosis, in 10 of which the alkalosis was due to bicarbonate therapy. The most striking case was a diabetic. This patient was admitted supposedly suffering from acidosis, since the urine contained a considerable amount of acetone. The small amount of alkali, however, given previous to admission, was sufficient to raise the P_H of the blood to 7.60. These workers also reported a case of cyclic vomiting in which a similar error in diagnosis had been made. Although the CO_2 content was not excessive, in this case 67 c.c., the P_H was 7.52. Three months later Ellis[3] reported 4 typical cases of alkalosis, in 2 of which the alkalosis was due to high-up intestinal obstruction. He has most excellently described the symptomatology of these cases. The following year Harrison and Perlzweig[7] described a case of uremia in which alkalosis developed without the administration of sodium bicarbonate. This patient had attacks of vomiting, and they believed this afforded the best explanation of the alkalosis. The CO_2 capacity and whole blood chlorides, however, were not notably abnormal, 69 c.c. and 486 mg. respectively, as found in cases of pyloric obstruction. The P_H was 7.6. Both Ellis[3] and Myers and Booher[8] have emphasized the fact that the kidneys may occasionally continue to secrete a strongly acid urine in certain renal conditions despite the fact that the P_H of the blood may be abnormally high. Apparently the kidneys are no longer able to excrete alkali readily.

Probably the most common causes of uncompensated alkalosis with alkali excess are the administration of sodium bicarbonate and high-up intestinal obstruction. It is apparent, however, from the cases referred to above that the etiology of uncompensated alkalosis is not always clear.

A number of cases are on record in which death, apparently from alkalosis, followed the administration of alkali. Uncompensated alkalosis following sodium-bicarbonate therapy would appear to be a condition to be guarded against, since we possess no therapeutic agent equal to bicarbonate in the treatment of acidosis. The most effective therapeutic agent in treating alkalosis is apparently ammonium chloride (Haldane[9]), the ammonia being converted to urea leaving the acidic ion. Hayden and Orr[10] have shown, however, that in intestinal obstruction sodium chloride is much more effective

than ammonium chloride. The reason for this is not entirely clear, although a suitable amount of sodium chloride does restore the blood chlorides to normal and relieve the toxemia.

Although it has been known for several years that over ventilation, either voluntary or due to oxygen want, would lead to a sufficiently increased blowing off of CO_2 to disturb the bicarbonate-carbonic acid equilibrium of the blood, Koehler[11] was the first to give a clear-cut demonstration of this in acute fevers. He has reported about 10 cases of uncompensated alkalosis due to CO_2 deficit. In one case, for example, the P_H was 7.60 and the total CO_2 47 c.c.

That deep roentgen-ray therapy, particularly when applied over the chest, pelvis and abdomen, results in an acute sickness has been known for some time, although the nature of the intoxication has been obscure. Hussey[12] was the first to show that uncompensated alkali excess followed x-ray exposure in rabbits. Myers and Booher[5] confirmed this in a human subject, and also noted a shift of the acid-base equilibrium of the blood to the alkaline side in a case of Hodgkin's disease undergoing radium therapy. More recently Doub, Bollinger and Hartman[13] have studied the acid-base balance in animals and in 150 patients treated with a modern deep therapy apparatus. A rapidly developing alkalosis and the continuation of this condition after large doses, have been shown by the determination of the P_H of the plasma and of the urine and by the use of indicators in the tissues. It is of interest that the reaction of the urine may remain alkaline for some time. Pagniez, Coste and Solomon[14] observed that alkalosis developed in nine out of twelve subjects in which 500 roentgen-ray units were applied to the spleen. In these cases the P_H seemed to be exclusively involved in the alkalosis.

There would appear to be no doubt then but that deep roentgen-ray therapy leads to an uncompensated alkalosis. Whether this is the underlying factor or one of several factors in the intoxication following x-ray therapy is not entirely clear.

The problem of uncompensated alkalosis is one deserving further careful investigation. In the meantime the clinician would do well to be quite as mindful of the occurrence and symptomatology of alkalosis as of acidosis. To be sure, the condition does not appear to be as common as acidosis, but it is more difficult to recognize and treat. Unfortunately a positive diagnosis of an uncompensated alkalosis cannot be made without data on the acid-base balance, and this necessitates observations on the P_H of the blood as well as on the bicarbonate. In the use of alkali therapy in the treatment of acidosis it should be borne in mind that the acid-base balance is occasionally quickly shifted from the acid to the alkaline side. Apparently the kidneys can no longer readily excrete alkali, since in such cases the reaction of the urine generally remains strongly acid.

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[14]Pagniez, Coste and Solomon: Compt. rend. Soc. de biol., 1925, xcii, 489.

—*V. C. M.*

Errata

In the article "Permanent Standards To Be Used with Benedict's Clinical Quantitative Test for Sugar in Urine" by Jeanette Allen Behre and William Muhlberg, June issue, the sentence in the twelfth line from the bottom of page 887 should read: One c.c. of urine, 3 c.c. of 0.2 per cent picric acid, 0.5 c.c. of 5 per cent sodium hydroxide and 4 drops of 50 per cent acetone (prepared fresh each day by dilution of acetone) are added in the order named, and the tube transferred at once to a boiling water-bath and heated for from ten to fifteen minutes, cooled and the contents diluted with water to 25 c.c.

The legend to Chart I, Heinekamp article, page 1069, August issue, should read: The full lines in this chart represent parasympathetic action and the broken line sympathetic.